NEURAL CONTROL OF THE PITUITARY GLAND

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by

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PREFACE

The suggestion of writing a monograph under the auspices of the Physiological Society was accompanied by the comments that such a monograph should— not be written like a review article giving an accumulation of all references but it should be a book which will be particularly useful for the advanced student in Physiology The following pages have therefore been based on a series of lectures given in the course on Physiology for the Part II of the Natural Science Tripos Cambridge University They have been written for the student who will within the course of a few years be standing on his own feet in the research field The other worker in mind has been the specialist in his own field who is interested in physiology in general For these reasons no attempt has been made to give a complete list of all the literature in this subject except in isolated instances as in Chapter 3 where the data have seemed to indicate a negative conclusion or to present the material from an unbiased point of view The attitude adopted has been rather to present the more important literature and the points of view most likely to be found true in the light of future work—as it seems to the author

I should like to express my sincere thanks to Mrs E M Collen for her invaluable help and advice in preparing the manuscript and to Mr H J Campbell for his skilled aid in preparation of many of the illustrations I am indebted to the following workers for their kind permission to reproduce various figures Professor Sir Wilfred Le Gros Clark F R S Professor G B Wislocki Professor E B Verney F R S Dr W J Atwell Dr P M Daniel Dr J D Green Dr Dora Jacobsohn Dr Marjorie M L Prichard Dr H Rydin Dr A Westman Dr G P Xuereb and to my co workers Drs K Brown Grant J G Gibson C von Euler S Reichlin and A C R Skynner and to the editors of the following journals the *Journal of Physiology* *Journal of Endocrinology* *Quarterly Journal of Experimental Physiology* *Research Publications of the Association for Nervous and Mental Disease* *American Journal of Anatomy* *Acta Pathologica Microbiologica Scandinavica* *Proceedings of the*

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usual eunuch sounds it no longer bothered about the hens nor fought with other cocks but rather kept a measured distance from them and showed altogether the nature of a true eunuch

In cocks c and f there was no sign of the testes in their usual place Combs and wattles grew again the birds retained their cock like nature crowed as before and also retained their former behaviour in respect of hens and other cocks These two cocks were killed on 30th January 1849 There was no sign of the testes in their normal place in cock c the testis was seen to have grown on the surface of the colon away from the back and bordered on both sides by the end of the caecum without however coalescing with the latter In cock f the same thing had occurred but the site of adherence was further back towards the middle of the caecum The testis was in each bird oval in shape 15 lines long 8 wide and 6 thick Strong branches of the mesenteric vessels went up to the testis penetrated in several places into the interior and could be traced to the seminal canals As I dis sected the testis a whitish milky liquid ran out which had exactly the same consistency and smell as normal cock semen Under the micro scope I recognized in this liquid very many small and large cells of 1/450–1/150 lines in diameter and in addition numerous spermato zoids with the most beautiful ciliary movements which increased considerably with the addition of a drop of water

The following general physiological results emerge from these investigations

(1) It is possible to transplant testes they heal up after they have been removed from the body the testis of one individual may be transplanted *into another and it can heal in the same place whence it* was removed or in quite a different place i e on the walls of the bowels

(2) The transplanted testis develops its characteristic features as a seminal organ even in a different place the seminal canals widen and become bigger and carry out their normal function while they secrete normal semen containing spermatozoids The same occurs here as in plants where the graft continues to grow with its own specific charac istics on the wild tree and brings forth its own fruit not that of the wild tree

(3) It is a known fact that severed nerves grow together again and that those parts the nerves of which have been cut regain feeling and movement when the nerves have healed That the nerve fibres which belong together do not always unite in such instances can be shown from the fact that skin from one part of the body can heal with that of another From the fact that a detached testis heals on to quite a different part of the body especially on to the intestine and continues to develop as a semen producing organ and secretes genuine semen it is obvious that there are no specific seminal nerves and this is one of the chief arguments against the existence of specific trophic nerves of which the sympathetic nervous system has until now been thought to *consist*

Royal Society the Philosophical Transactions of the Royal Society and *the Journal of Anatomy (London)*

I am greatly indebted to my wife for her patience and help

In conclusion I should like to give the translation (for which I am grateful to Miss J L Shadwell) of a paper which may be taken as a model for the following reasons (a) it is the first paper of any importance in the field of endocrinology, (b) it is brief and concise (c) the work recounted is beautifully planned and economical in the use of animals and (d) the results are clear

Transplantation of the testes (*Arch Anat Physiol* 1894) by Professor Berthold Gottingen

On August 2nd this year I castrated six young cocks i e a b c of three months and d e f of two months In some of these birds the wattle the comb or the spurs were removed Both testes were removed from a and d these birds later showed the typical nature of the eunuch behaved in a cowardly way, only occasionally briefly or without energy fought with the cocks and emitted the well known monotonous sounds of the eunuch Comb and wattle became pale and developed only slightly the head remained small When these birds were killed on December 20th an insignificant hardly perceptible cicatrix was found in place of the testes The seminal conductors were seen to be thin delicate little fibres

Cocks b and e were castrated in the same way but only one testis was removed from the body the other remained isolated in the abdominal cavity In cocks c and f however both testes were removed from the abdominal cavity and then one testis of cock c placed in the abdominal cavity of cock f and one testis of cock f in the abdominal cavity of cock c in the bowels

The four cocks (b e c f) showed in their general behaviour the nature of uncastrated birds they crowed quite audibly were often fighting among themselves and other young cocks and showed the usual friendly interest for the hens also their combs and wattles developed normally

Cock b was killed on October 4th the (one) testis had healed in its original place had increased by more than half in size numerous blood vessels were present the seminal canals were very clear and on cross section emitted a whitish liquid containing large and small cells but no spermatozoids

The fairly well developed combs together with the wattles were removed from cocks c e f and the abdominal cavity opened on the same day in order to examine the testis In cock e I found the testis in the normal place as in the dead cock b I detached it drew it out of the abdominal cavity and found it to be similar to that of cock b The abdominal wound soon healed comb and wattle cicatrized but did not grow again Instead of crowing as before the animal only made the

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(4) The remarkable consensual and antagonistic interaction of individual and communal life which develops at puberty and persists into an advanced age is also evident when the testes are removed from their original place and from their nerves and have healed on to quite a different part of the body With regard to voice reproductive instinct, love of fighting growth of the comb and wattle these birds remain true cocks As the testes transplanted to a different place can no longer be connected with their original nerves and as there are no specific nerves directing secretion as is clear from paragraph 3 it follows that the consensus in question is conditioned by the secretion of the testes i e by their action on the blood and then by the consequent action of the blood on the whole organism of which it must be admitted the nervous system forms a very substantial part

Arch Anat Physiol 1849 42-46

CHAPTER 1

INTRODUCTION

Meaning of the term hormone

The first demonstration of an internal secretion is to be found in the clear and precise experiments of Berthold in 1849 The significance of this work was overlooked for many years until Bayliss and Starling reported their results regarding secretin in 1904 They emphasized the fact that a specific chemical substance may be produced in an organ or tissue may be liberated into the blood stream and carried to excite another organ situated at a distance Such substances are essentially chemical messengers and in searching for a name to characterize them this point was kept in mind Eventually Mr W B Hardy proposed hormone derived from ὁρμάω (I arouse to activity) and although the property of a messenger is not suggested by this term it was finally adopted (see Starling 1905 and Bayliss 1915)

An essential feature of a hormone is the fact that it is secreted into and carried by the blood stream Nowadays there is some need for another term to represent the precursor of a hormone as it exists in the gland of origin This precursor may or may not be the same chemical substance as the hormone itself but a separate term would emphasize the dual nature of hormonal formation and secretion An endocrine gland (such as the thyroid) subtracts simple substances from the blood stream and builds them into more complex compounds which are stored in the gland for a variable period This store is drawn upon according to the requirements of the organism In many cases it seems that a mother molecule is split into simpler fractions or changed in some way before being liberated into the blood This self evident sequence of events is mentioned here for two reasons Firstly the hormone content of a gland clearly varies with both the rate of formation and the rate of liberation of the hormone into the blood Therefore the hormone content of a gland by itself gives no indication of the activity of the gland Secondly the answer to the question— How many hormones does a particular endocrine gland produce? —may depend on whether the

out also that there is not the same tendency to hypoglycaemia after stalk section as after hypophysectomy

It may be said then that some glands although atrophic retain a residuum of activity when deprived of the main stimulus to their normal function though others such as the posterior pituitary gland become completely inactive

(*b*) *Normal activity* The central nervous system is ultimately responsible for maintaining the activity of most endocrine glands at normal levels and to a large extent for regulating their activity according to the requirements imposed by a varying environment

Inter relationship between the nervous system and endocrine glands

In general a reciprocal relationship exists between the central nervous system and the endocrine glands Firstly the nervous system regulates directly or indirectly the functional activity of the adenohypophysis neurohypophysis ovaries testes thyroid adrenal cortex and adrenal medulla Secondly the hormones derived from these glands or the actions they exert peripherally react back on the nervous system and thereby produce a variety of neurological effects

(*a*) *Neural control of endocrine glands* In 1925 Starling and Verney described two extreme methods used in physiological investigation firstly the synthetic method whereby a particular organ is studied in isolation the normality of the environment being sacrificed to obtain simplicity and a higher degree of experimental control and secondly the analytic method whereby the organ is studied in a relatively intact animal thus sacrificing much control of the variable conditions in order to obtain a more normal environment Most organs and systems were first studied in the more or less isolated state and their intrinsic properties investigated before being studied in the more intact animal in which their functions relative to other systems and relative to the animal as a whole were matters of concern Much is now known regarding the anatomy and histology of the endocrine glands and the biochemistry and physiological properties of the compounds they secrete but still only little is known as to the manner in which the activity of the different glands is co ordinated with that of the other systems of the body or the way in which this activity is varied according to changes in the environment It is

term hormone is taken to mean the active principles stored in the gland or more correctly, the principles as liberated into the blood stream The case of the adenohypophysis (anterior pituitary gland) is in this respect obscure Six hormones' are now generally ascribed to this gland on the grounds that it is possible to prepare six extracts from the gland with distinct physiological activities However it is not certain that these so called hormones are liberated as such into the blood It may be that only two or three compounds pass from the adenohypophysis into the blood but that these compounds possess two or more of the activities usually attributed to the six hormones The ultimate test by which the number of substances secreted by a given endocrine gland will be decided is the qualitative and quantitative analysis of the hormones in the venous blood from the gland The time when such tests are practical and accurate seems to be approaching for some glands such as the adrenal medulla and cortex, but it seems still remote for others such as the adenohypophysis

Levels of endocrine activity

(*a*) *Autonomous activity* When deprived of their physiological stimulus the level of activity of the various glands becomes markedly decreased or disappears entirely Thus the denervated neurohypophysis ceases to secrete and undergoes atrophy and the denervated adrenal medulla shows a very reduced activity and becomes relatively inexcitable to direct electrical stimulation In hypophysectomized animals the ovaries and testes atrophy and reproductive activity ceases although the initial stages of oogenesis and spermatogenesis still occur in some species such as the rat the adrenal cortex atrophies though some secretory action sufficient to maintain life is maintained and thyroid activity is greatly reduced though not abolished Similarly if the anterior pituitary is deprived of its physiological stimulus that is if the pituitary stalk is cut or if the gland is transplanted to a site remote from the sella turcica then gonadal atrophy occurs growth ceases and the thyroid and adrenal cortex undergo atrophy Since the reduction in activity of the thyroid and adrenal cortex is not so complete according to some accounts in the pituitary transplanted as in the hypophysectomized animal it appears that residual secretion of thyrotrophic and adrenocorticotrophic hormones still occurs It might be pointed

relating the hypothalamus functionally with these glands as well as with the neurohypophysis and adrenal medulla The greater part of what follows (Chs 2–12) is an attempt to analyse the

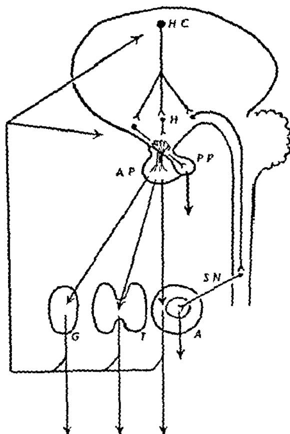


Fig 1 Diagram to illustrate the reciprocal relationship between the central nervous system and endocrine system
A adrenal gland *A P* anterior pituitary *G* gonads *H* hypothalamus *H C* higher centres *P P* posterior pituitary *S N* splanchnic nerves *T* thyroid

mechanism by which the central nervous system and the hypothalamus in particular controls and integrates the activity of these glands

(*b*) *Hormonal control of the nervous system* It is clear that the interplay between the central nervous system and endocrine

clear that environmental factors exert a profound effect on the secretory activity of these glands but the mechanism by which these effects are produced is known only in outline For example trauma to a limb may result in a flexor reflex response in that limb and also to a discharge of hormones from the adrenal cortex Much could be said of the sequence of events starting in trauma and ending in contraction of the flexor muscles but until recently very little could be said about the mechanism linking trauma and discharge of the adrenal cortical hormones The synthetic approach has been studied in detail but the analytic method has hardly been applied to the glands of internal secretion

It is clear that the central nervous system is largely responsible for correlating endocrine activity with that of the other systems of the body and with the varying requirements of the organism due to environmental change In the case of two endocrine glands the neurohypophysis and the adrenal medulla it is easy to understand how such control can be mediated for both of these glands are richly innervated The neurohypophysis receives a direct nerve supply from the hypothalamus known as the hypothalamo hypophysial tract whilst the adrenal medulla is connected to the hypothalamus by descending tracts in the brain stem and spinal cord and by the final supply which passes in the splanchnic nerves and lumbar sympathetic chain On the other hand the control exerted by the nervous system over the adenohypophysis thyroid adrenal cortex ovaries and testes has been difficult to understand These endocrine glands are clearly under neural control and yet they have a very scanty innervation if any at all The few nerve fibres that have been traced to some of these glands are in all probability vasomotor in nature and not *secreto motor* The problem as to how these glands are influenced by nervous processes in the absence of a secreto motor nerve supply would be clarified if it could be found how the adenohypophysis is brought under neural control for the thyroid adrenal cortex and gonads would then be brought indirectly under similar control via the action of the anterior pituitary eutrophic hormones There is now much evidence that the hypothalamus controls the activity of the adenohypophysis and thereby the gonads, thyroid and adrenal cortex through the hypophysial portal vessels of the pituitary stalk and some such sequence of events as illustrated in Fig 1 may be envisaged as

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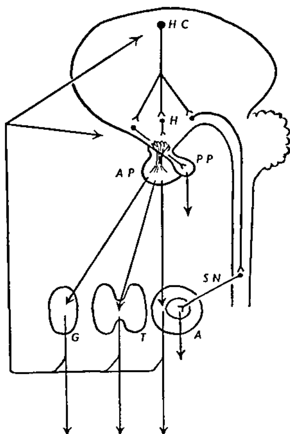


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CHAPTER 2

NERVE SUPPLY AND BLOOD SUPPLY OF THE ADENOHYPOPHYSIS (ANTERIOR PITUITARY GLAND)

In discussing questions involving the detailed anatomy of the pituitary gland the standardized nomenclature suggested by Rioch Wislocki and O Leary (1940) will be used (Fig 2) The *neurohypophysis* is described by these authors as consisting of three parts—the *median eminence of the tuber cinereum* the *infundibular stem* and the *infundibular process* (lobus nervosus or neural lobe) The first two parts may be collectively referred to as the *infundibulum* or *neural stalk* The *adenohypophysis* or *lobus glandularis* is likewise divided into three parts—the *pars tuberalis* the *pars intermedia* and the *pars distalis* The neural stalk together with its sheath of portions of the lobus glandularis is designated the *hypophysial stalk*

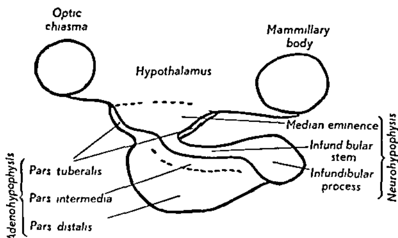


Fig 2 Diagram of a sagittal section through the pituitary gland of a rabbit to illustrate the terminology of Rioch Wislocki & O Leary 1940

glands (at least the adrenal cortex thyroid ovaries and testes—the so called pituitary target organs) is one of reciprocity (see Fig 1) The target organ hormones seem to exert two main actions on the central nervous system By means of a type of feedback mechanism they appear to exercise a fine control over their own secretion For example a rise in the blood oestrogen level depresses pituitary gonadotrophic secretion and so ovarian secretion of oestrogens Whether this effect of a rise in concentration of a target organ hormone in the blood is due to an action on some cerebral mechanism such as the hypothalamus which in turn affects pituitary secretion or to a direct action on pituitary cells or both is uncertain—though the former possibility should be kept in mind However the secretion of a particular trophic hormone is affected by other factors as well and the final pituitary output seems to depend on the summation of effects produced by many stimuli both humoral and neural

Another effect of circulating hormones on the central nervous system results in modification of the behavioural reactions of the animal As examples of this action might be quoted the psychological changes that occur throughout the different phases of the sex cycle and the effect of disturbances in the blood level of the thyroid hormone A pinch too little of thyroxine spells idiocy a pinch too much spells raving delirium (Hoskins 1941) It is very likely that such changes in the overt pattern of behaviour are directly due to the influence of the various hormones on the central nervous system but little work has been performed in an attempt to analyse the detailed mechanism by which such effects are produced This subject is discussed further in Chapter 13

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observed nerve fibres leaving the sympathetic plexuses around the vessels of the circle of Willis and passing down the pituitary stalk with the arterial supply to the gland Hair (1938) working on the cat likewise found a rich innervation passing with the vessels of supply to the gland as did Truscott (1944) who studied the rat Rasmussen (1938) in a detailed study of the innervation of the human hypophysis supplemented by observations on the rat guinea pig rabbit cat dog and monkey found some sympathetic fibres passing from the cavernous plexus to the pars distalis but in view of the fact that large areas of the pars distalis were found to be free of nerve fibres he drew the conclusion that these fibres were most likely connected with the vascular system Green (1951*a*) studied the innervation of the pituitary gland in seventy five species of vertebrates from cyclostomes to man and found perivascular fibres in the pars tuberalis but not in the pars distalis of the hypophysis

It may then be taken as established that sympathetic nerve fibres pass to the pituitary gland from the surrounding perivascular plexuses but it is not yet clear whether the main secreting mass of the adenohypophysis the pars distalis receives any of these fibres or what the nature (secreto motor or vasomotor) of such fibres might be It is very likely that the sympathetic innervation is destined mainly for the pars tuberalis and that the pars distalis receives few if any fibres This view is in accordance with that expressed by Green (1951*a*) after his comprehensive investigation of the problem and it would indicate that the fibres are vasomotor in nature for the pars tuberalis is the most vascular region of the pituitary gland and as far as is known has no definite endocrine function

Experimental data concerning the relationship between the cervical sympathetic system and anterior pituitary function is clear in its implications Although many fragmentary claims have been made that stimulation or removal of the cervical sympathetic chain affects the secretion of gonadotrophic or thyrotrophic hormones the fact that complete sympathectomy does not prevent normal reproduction in female cats (Cannon Newton Bright Menkin and Moore 1929) and does not cause any very significant change in the metabolic rate of cats (Cannon et al 1929) or rats (Lee and Bacq 1933) demonstrates that a sympathetic innervation of the pituitary plays no appreciable part in the con-

Major Divisions and Subdivisions of the Mammalian Hypophysis
(after Rioch Wislocki and O'Leary, 1940)

Adenohypophysis	*Lobus glandularis*	1 *Pars distalis*	anterior lobe
		2 *Pars tuberalis*	
		3 *Pars intermedia*	posterior lobe
Neurohypophysis	*Lobus nervosus (neural lobe)*	1 *Infundibular process*	posterior lobe
	Infundibulum (neural stalk)	1 *Infundibular stem*	Neural stalk together with sheath of portions of lobus glandularis designated as *hypophysial stalk*
		2 *Median eminence of the tuber cinereum*	

The nerve supply and blood supply of the anterior pituitary gland or more accurately the adenohypophysis refers to the supply of the three parts of the pituitary gland which are derived from Rathke s pouch of the embryo

The Nerve Supply of the Adenohypophysis

Over a hundred years ago a nerve supply to the pituitary gland originating from the sympathetic plexus around the carotid artery was described by Bougery (1845) He suggested that the pituitary gland formed a link between the brain and the sympathetic nervous system that is between the mind and the viscera Since the time of Bougery and especially since about 1920 when light was first thrown on the hormonal activities of the adenohypophysis by Philip Smith H M Evans and others the nerve supply of this part of the pituitary has been the subject of investigation by many workers It has been suggested that it receives secreto motor nerve fibres from three sources—sympathetic fibres from the plexus around the internal carotid artery parasympathetic fibres from the petrosal nerves and hypothalamic fibres from the median eminence and infundibular stem [For further references to the literature on this subject see Harris (1948)]

Sympathetic supply

Dandy (1913) was the first to give a detailed description of the sympathetic pathway to the gland He used the intravitam methylene blue method of staining these fibres in the dog and

series of papers from the laboratory of Shelesnyak Zacharias and Rosen describing an immediate pseudopregnancy response (a) in 50 per cent of rats that had had the nasal mucosa (which is innervated by the sphenopalatine ganglion) anaesthetized with nupercaine (b) in 40 per cent of rats that had had the spheno palatine ganglion extirpated and (c) in 100 per cent of rats that had had the Vidian ganglion removed However these data again appear to be of doubtful significance in respect to pituitary innervation for the same workers found that removal of the sphenopalatine ganglion does not result in any abnormality of genital development or reproductive capacity in the rat and Friedgood and Bevin (1941) found pseudopregnancy occurs in 64 per cent of rats following bilateral removal of the superior cervical ganglion in 39 per cent following bilateral cervical sym pathectomy and in 23 per cent following blank neck operations It appears that a state of pseudopregnancy in the rat may follow a wider variety of sensory stimuli (trauma) than was previously thought Hair and Mezen (1939) and Vogt (1942) also demon strated that the greater superficial petrosal nerves are probably of little importance for anterior pituitary function when they found that the reflex release of gonadotrophic hormone which normally follows coitus in the rabbit still occurs after bilateral avulsion of the facial nerve and geniculate ganglion or after the destruction of the petrosal nerves at the geniculate ganglion

There is no sound evidence that a parasympathetic innervation of the anterior pituitary gland plays any part in regulating the activity of this gland

Hypothalamic supply

Cajal (1894) was the first to describe the nerve fibres which pass from the hypothalamus down the pituitary stalk to the pituitary gland The anatomy of the hypothalamo hypophysial tract will be dealt with in more detail when discussing the inner vation of the neurohypophysis for it is in this part of the pituitary that by far the greater number of nerve fibres terminate However even in the first description by Cajal it was mentioned that a few of these fibres cross the boundary zone between the neuro and adeno hypophysis and terminate in the pars inter media

trol of secretion of the gonadotrophic and thyrotrophic hormones The observations of Phillips (1942) that action potentials may be recorded from the pars distalis during electrical stimulation of the cervical sympathetic trunk of Collin and Hennequin (1936*a* and *b*) and of Popjak (1940) that histological changes in the pituitary and hypothalamus follow removal of the superior cervical ganglion might be explained on a sympathetic vasomotor innervation of the hypophysial vessels Two easily observable reactions that have been much used in investigating the effects of nervous stimuli on pituitary secretion are the pseudopregnancy response of the rat and the ovulation response of the rabbit both elicited by sterile mating or some form of artificial stimulation There is much evidence that in these reactions the anterior lobe of the pituitary gland is activated by a nervous reflex Some workers have found that partial sympathectomy in the rat abolishes or reduces the pseudopregnancy response to artificial stimulation of the vagina or uterine cervix and others (Friedgood and Cannon 1936) have produced maturation of ova in the rabbit by electrical stimulation of the cervical sympathetic system But again it is possible that these minor changes are due to vasomotor effects since pseudopregnancy still follows sterile coitus in the partially sympathectomized rat (Vogt 1933 Friedgood and Bevin 1938) and ovulation still follows sterile coitus in the partially or completely sympathectomized rabbit (Haterius 1934 Brooks 1935) There is no sound evidence that a sympathetic innervation of the anterior pituitary gland plays any part in regulating the activity of this gland

Parasympathetic supply

In 1932 Cobb and Finesinger and Chorobski and Penfield described a parasympathetic innervation of pial vessels in the cat and monkey Nerve filaments were found to run from the greater superficial petrosal branch of the facial nerve to the carotid plexus and these were described as carrying vasodilator fibres Following this discovery Hinsey and Markee (1933) suggested that secreto motor fibres might reach the anterior pituitary gland over a similar pathway and Zacharias (1941) reported that in the rat a branch from the Vidian ganglion situated at the junction of the greater superficial and great deep petrosal nerves runs to at least the capsule of the pituitary gland There then followed a

tive tissue septum separates the neural lobe and anterior lobe This septum therefore limits the possible pathway by which hypothalamic nerve fibres could reach the pars distalis Such fibres would have to traverse the relatively small region of the pars tuberalis and zona tuberalis (anterior pole of the pars distalis) Sections of the zona tuberalis were therefore stained by Bodian s method for nerve fibres and by Gomori s method for reticular fibres and as a control procedure sections of a rabbit s brain were mounted on the same slides and stained simultaneously The results are shown in Figs 3–6 and it may be seen that Bodian s stain impregnated the nerve fibres in the brain but that none are visible in the pituitary tissue stained on the same slide whilst the Gomori method revealed reticular fibres in the pia arachnoid meninx on the surface of the brain and a multitude of reticular fibres amongst the cells of the zona tuberalis also stained on the same slide It seems very probable that the prominent bundles of nerve fibres seen by Vasquez-Lopez (1949) passing from the hypothalamo hypophysial tract to the pars distalis in the rabbit were reticular fibres around the portal vessels This conclusion is supported by the careful work of Wingstrand (1951) on the avian pituitary gland Wingstrand used a variety of silver stains and controlled the interpretation of any fibre pattern seen in the pars distalis by staining the tissue also for reticular fibres He concludes In perfectly impregnated slides in which the reticular fibres are unstained the adenohypophysis contains no or very few nerve fibres There is nowadays so much evidence that the pars distalis does not receive prominent bundles of nerve fibres that any such claim of a rich hypothalamic innervation requires the substantiation of rigid control procedures In particular sections from a normal gland and a denervated gland (after pituitary stalk section and removal of the superior cervical sympathetic ganglia) stained on the same slide should help to clarify the nature of any fibres seen in the pars distalis

The fibre components of the neurohypophysis and adenohypophysis have been studied by Green (1951*b*) using phase microscopy It was found that the nerve fibres known to be present in the neurohypophysis were not visible in media of high refractive index whereas raising the refractive index of the media did not appear to diminish the number of fibres seen in

Pars intermedia Many workers have confirmed the existence of nerve fibres entering the pars intermedia from the infundibular process In most accounts these fibres are described as being scanty (Rasmussen 1938 Green 1951a) The functional significance of this innervation is unknown since the only hormone known to be secreted by the pars intermedia is the melanophore expanding hormone, and since the pars intermedia is a variable part of the vertebrate pituitary gland being absent in such forms as birds the armadillo elephant cetacea and very poorly developed in man

Pars tuberalis This highly vascular part of the adenohypophysis which is stretched out as a thin collar of tissue encircling the median eminence of the tuber cinereum also receives nerve fibres from the hypothalamo hypophysial tract These fibres are more common than those passing to the pars intermedia and although no very close relationship of nerve terminals to cells has been seen an intimate association with the blood vessels of the region has been noted (Green & Harris 1947 Green 1951a)

Pars distalis The innervation of the pars distalis has been a much debated subject though the position now seems to be clarified The pars distalis is the part of the adenohypophysis responsible for secreting the gonadotrophic thyrotrophic adrenocorticotrophic lactogenic and growth hormones so that the problem of a pars distalis innervation is one of much significance Although some workers have described the passage of numerous fibres from the hypothalamo hypophysial tract to the pars distalis the majority have either found a complete absence of such fibres or the presence of so few that it seemed very unlikely that they constitute a secreto motor innervation for the gland (Rasmussen 1938) Green (1951a) in his survey of the pituitary glands of many types of vertebrates concludes In none of the animals studied has an innervation of the pars distalis been found

It is likely that the conflicting statements in the older literature were due to the ease with which reticular connective tissue fibres may be mistaken for nerve fibres when a silver stain is used Reticular fibres are prominent amongst the cells of the pars distalis and also exist around the portal vessels at least in the human (Green 1948) and probably in other forms Since the portal vessels start amongst the nerve fibres of the hypothalamo hypophysial tract and pass into the pars distalis any reticular fibres in the sheaths of these vessels may be easily mistaken for a nervous pathway to the gland unless the staining technique is carefully controlled With this point in mind the pars and zona tuberalis of the whale s pituitary gland was examined (Harris 1950a) The gland of the whale was chosen since in this form a thick connec

the pars distalis From this study it is possible to conclude that the fibres seen in the pars distalis were different in nature from the nerve fibres of the neural lobe

In conclusion it may be said that the available evidence indicates that the pars distalis receives very few if any nerve fibres

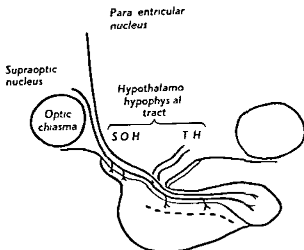


Fig 7 Diagram of a sagittal section through the pituitary gland

The hypothalamo-hypophysial tract comprising the supraopticohypophysial (*S O H*) and tuberohypophysial (*T H*) tracts innervate the neurohypophysis A few nerve fibres may be seen entering the pars tuberalis and pars intermedia but it is doubtful if the pars distalis receive any innervation

THE BLOOD SUPPLY OF THE PARS DISTALIS (ANTERIOR LOBE)

The blood supply of the anterior pituitary may be compared in a general way with that of the liver Both organs have a systemic arterial supply a portal blood supply and a systemic venous drainage (Fig 8) The vascular supply of the pars distalis is separate from that of the neural lobe in sections this is clearly seen since these two vascular fields are separated by the relatively avascular pars intermedia

Systemic arterial supply and venous drainage

The anatomy of the arteries supplying the anterior lobe of the pituitary may be studied by a variety of methods Methods involving dissection injection with indian ink dyes or other



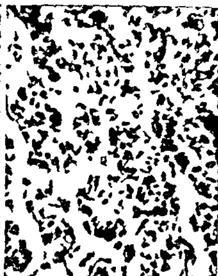


Fig 3 Fig 4

Microphotogr ph of a section through a rabbit s brain (Fig 3) stained to show nerve fibres on the same slide as the section through the zona tuberalis of the anterior pituitary of a whale (Fig 4)

Note the presence of well stained nerve fibr s in the brain tissue The pituitary tissue hows faintly stained vascular endothelium but an absence of any definite nerve fibre Formol fixation 10 μ thick sections Bodian s stain × 75





Fig 5 Fig 6

Microphotograph of a section through a rabb t s brain (Fig 5) stained to show reti ular connecti e tissue fibres on the same slide as the section through the anterior pituitary of a whale (Fig 6)

Note the absence of reticular fibres in the bra n though they are present n the pia arachnoid men nx on the surface of the brain Many reticular fibres exist around clusters of glandular cells in the p tuitary Formol fixation 10 μ thick sections Gomori s stain × 275 (Figs 3–6 Harris C W (1950) *J Physiol* 111 361)

vessels of the opposite side forming an arterial ring around the neural lobe Branches springing from this arterial ring supply the neural lobe and part of the infundibular stem Xuereb et al could find no branches of either the superior or inferior hypophysial arteries supplying the pars distalis This study is particularly opportune at the moment in view of the operation of hypophysectomy which is being performed in the human for extensive carcinoma of the breast The systemic supply to the anterior lobe in other forms may vary considerably from the human pattern Dandy and Goetsch (1911) described the supply for the pars distalis of the dog as being derived from a large

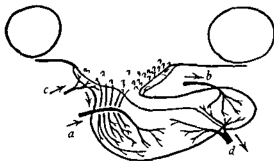


Fig 8 Diagram of a sagittal sect on through the pituitary gland of a rabbit illustrating the hypophysial blood supply

The anterior and poster or hypophysial arteries *a* and *b* are derived from the internal carotid arteries The arterial twigs *c* to the pars tuberalis plexus (which in turn supplies the primary plexus of the portal vessels) are derived from the internal carot d and posterior communicating arteries The venous drainage *d* passes to surrounding venous sinuses in the dura mater or in the basisphenoid bone

number of branches of the circle of Willis which pass down the pituitary stalk A similar supply for the pars distalis of the monkey was mentioned by Wislocki (1938) viz several small branches from the carotid arteries or circle of Willis which pass through the leptomeninges and penetrate the anterior lobe of the hypophysis in the vicinity of the neck (Fig 9) In the rabbit (Harris 1947) the systemic artery to the pars distalis is a branch of one internal carotid usually the left arising before the carotid makes its intracranial exit from the dura mater (Fig 8) Since the point of origin of this artery is below the plane of the diaphragma sellae this vessel passes immediately into the fibrous

masses followed by dissection or histological study staining the vessel walls or contained blood and sectioning for microscopy have all been used A method that demonstrates the general arrangement well is to inject the arterial tree with a mixture of indian ink rice starch and gelatin and then to dissect and follow the vessels under a binocular microscope In this way the main arterial twigs can be seen black against a pale background since the rice starch granules do not allow the perfused mixture to penetrate capillaries This method was used by Langley (1925) to investigate the course of the blood of the renal artery

The systemic arterial supply of the pars distalis consists of one or more small arteries derived from the internal carotid artery or the circle of Willis (posterior communicating artery) The exact pattern of these arterial twigs is variable from species to species and from one animal to another in the same species In some forms these vessels are absent and then the whole blood supply to the pars distalis is derived from the hypophysial portal system

Most of the earlier studies of the arterial supply were made in the human (Luschka 1860 Peremeschko 1867) and substantiated by the later findings of Fuchs (1924) Popa and Fielding (1930) and others According to these workers the supply in man is derived from two or more arteries passing from the internal carotid as it lies in the cavernous sinus or frequently after leaving the sinus These vessels penetrate the posterior superior angle of the gland at the junction of the pars distalis and neural lobe and accompanied by a certain amount of connective tissue pass anteriorly in the gland More recent and more accurate studies of the arterial supply in man have been made by McConnell (1953) and Xuereb Prichard and Daniel (1954*a*) Xuereb et al studied the vessels of specimens injected with neoprene latex or Berlin blue and described two branches of the internal carotid artery the superior and inferior hypophysial arteries on either side which pass to the gland Each superior hypophysial artery distributes branches to the hypophysial stalk and gives off a substantial branch the artery of the trabecula which courses through the pars distalis without giving off any branches to this part of the gland to supply the lower part of the infundibular stem Each inferior hypophysial artery divides into a medial and lateral branch These branches anastomose with the corresponding

capsule of the pituitary and is carried into the anterior part of the gland with the connective tissue that separates the zona tuberalis from the rest of the pars distalis In the pig and possibly in other ungulates the arterial supply to the anterior lobe seems to arise from the rich arterial plexus the rete mirabile which surrounds the pituitary gland The hypophysial vessels of the rat were described by Landsmeer (1951) who found the pars distalis of the rat receives only a scanty arterial supply through a very fine branch of the caudal hypophysial artery The vascular system of the pituitary has been investigated in other mammals (mouse and guinea pig Morin and Bottner 1941 cat Nowakowski 1951) Wingstrand (1951) has found that the pars distalis of birds receives no systemic arterial supply and that the entire blood supply of the anterior lobe of the pituitary is derived from the hypophysial portal system

From the above description it may be seen that the arterial supply varies considerably in different forms and this fact is of importance in various surgical procedures For example in experiments involving section of the pituitary stalk it is important to know whether the arterial supply is derived from the internal carotid artery below the level of the diaphragma sellae as in the rabbit in which case it lies safely out of the plane of stalk section or whether it is derived from the internal carotid artery or circle of Willis and descends along the stalk In rats and birds where the major or entire blood supply of the pars distalis is derived from the hypophysial portal vessels any experiment involving stalk section requires careful control procedures to verify that the anterior lobe tissue is viable and has acquired a good blood supply from other sources

The venous drainage of the pars distalis is by means of short wide veins draining into venous sinuses situated around the gland or inferiorly in the sphenoid bone [Drainage into a sphenoidal venous sinus is of importance in experiments in which the pituitary is exposed by a parapharyngeal approach In the rabbit for example such exposure entails obliteration of the sphenoidal venous sinus and is followed by retrograde thrombosis of vessels in the pars distalis and of the hypophysial portal vessels] Fig 9 shows the general findings of Wislocki in the monkey and it seems that a similar arrangement holds for the venous drainage of the anterior lobe in other mammals and birds

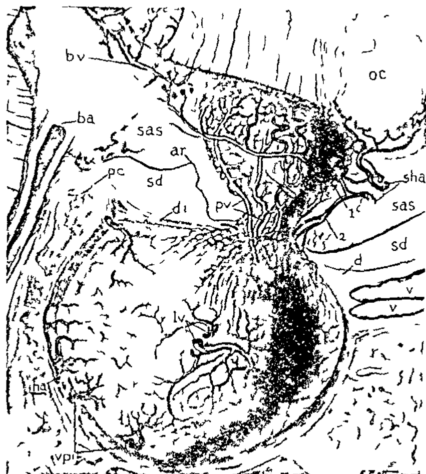


Fig. 9. Schematic drawing of the hypophysis of the adult rhesus monkey. *a*, arachnoid membrane; *ba*, basilar artery; *bv*, basilar veins; *d*, dura; *di*, sellar diaphragm; *ha*, inferior hypophysial artery; *lv*, lateral hypophysial veins; *oc*, optic chiasma; *pc*, posterior clinoid process; *pv*, portal venules; *sas*, subarachnoid space; *sd*, subdural space; *sha*, superior hypophysial arteries (1, branches to hypophysial stalk; 2, branches to anterior lobe); *v*, dural vein; *vpi*, veins of infundibular process.
(From Wislocki, G. B. (1938) *Res. Publ. Ass. nerv. ment. Dis.* 17, 48.)

capsule of the pituitary and is carried into the anterior part of the gland with the connective tissue that separates the zona tuberalis from the rest of the pars distalis In the pig and possibly in other ungulates the arterial supply to the anterior lobe seems to arise from the rich arterial plexus the rete mirabile which surrounds the pituitary gland The hypophysial vessels of the rat were described by Landsmeer (1951) who found the pars distalis of the rat receives only a scanty arterial supply through a very fine branch of the caudal hypophysial artery The vascular system of the pituitary has been investigated in other mammals (mouse and guinea pig Morin and Bottner 1941 cat Nowakowski 1951) Wingstrand (1951) has found that the pars distalis of birds receives no systemic arterial supply and that the entire blood supply of the anterior lobe of the pituitary is derived from the hypophysial portal system

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Hypophysial portal vessels

(1) *Historical* Many years ago Professor Fr I Rainer of Bucharest noticed prominent blood vessels running along the human pituitary stalk (cf Fig 20) and suggested to one of his pupils (later Professor G T Popa) that a more detailed study should be made of these vessels Popa succeeded in making serial sections through the human gland still attached to the dien



Fig 10 Photomicrograph of a transverse section through the median eminence of a rat

Blood vessels injected with indian ink *B* base of skull and meninges *H* hypothalamus *M E* median eminence showing the tuft of capillary loops constituting the primary plexus of the portal vessels *P T* pars tuberalis *V* third ventricle ×70

(From Green J D & Harris G W (1947) *J Endocrinol* 5 136)

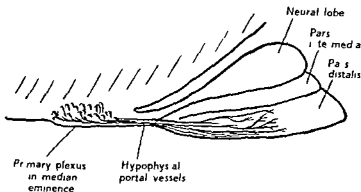
cephalon by the pituitary stalk He observed that the prominent vessels of the stalk were in reality portal vessels in that they ended in capillaries if traced upwards to the tuber cinereum or if traced downwards into the hypophysis These observations remained unpublished until at the suggestion of Professor G Elliot Smith the anatomy of these vessels was reinvestigated at University College London Popa and Fielding (1930 1933) then made their first reports on these structures that became known as the hypothalamo hypophysial portal vessels In these



Fig 11 Photomicrograph of a horizontal sect on through the hypothalamus and pituitary gland of a rat

Blood vessels injected with indian ink Note the small arterial twigs (*A*) which supply the pars tuberalis (*P T*) and its associated vascular plexus and thus the loops of the median eminence The portal vessels (*P V*) are seen running from the pars tuberalis and median eminence along the ventral surface of the infundibular stem to the pars distalis (*P D*) ×15

(From Green J D & Harris G W (1947) *J Endocrinol* 5 136)



F g 12 D agram of a midline sag ttal section through the hypothalamus and p tu tary gland of the rat To illustrate the general d sposit on of the hypophysial stalk and portal vessels

original accounts of Popa and Fielding and Wislocki were that Wislocki on histological evidence drew the conclusion that blood flowed in these vessels in a direction *towards* the pituitary gland and that the lower end of the portal vessels connected with the capillaries of the anterior lobe of the pituitary only and not with the capillaries of the neural lobe Little more work was performed

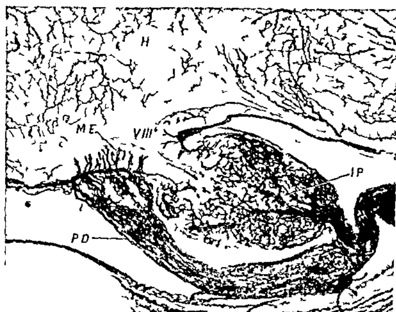


Fig 16 Photomicrograph of a sagittal section through the hypothalamus pituitary gland and related structures of a dog
Blood vessels injected with indian ink *H* hypothalamus *I P* infundibular process *M E* median eminence with contained sinusoidal loops *P D* pars distalis *V III* third ventricle × 26
(From Green J D & Harris G W (1947) *J Endocrinol* 5 136)

on the anatomy of these vessels during the next ten years until the suggestion was made that they might form part of a mechanism whereby the hypothalamus regulates anterior pituitary activity Green and Harris (1947) then reinvestigated the region in a variety of common laboratory mammals and largely confirmed the findings of Wislocki

(ii) *The anatomy of the hypophysial portal vessels in mammals* In mammals the hypophysial portal system seems to be arranged

as follows (see Fig 8) Small arterial twigs from the internal carotid arteries and posterior communicating arteries run to supply a rich vascular plexus situated in the pars tuberalis of the pituitary gland From this plexus arises a multitude of capillary loops or tufts which penetrate into the tissue of the median eminence and there come into intimate relationship with the

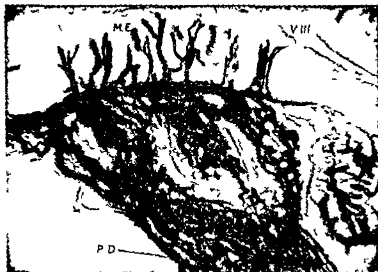


Fig 17 Photomicrograph through the median eminence of a dog Blood vessels injected with indian ink *M E* median eminence containing characteristic vessels *P D* pars distalis *V III* third ventricle × 66 (From Green J D & Harris G W unpublished)

nerve fibres of the supraopticohypophysial, tuberohypophysial and other nerve tracts These loops or tufts are collectively referred to as the *primary plexus* of the hypophysial portal vessels One criterion of the limits of the median eminence is that it is that part of the tuber cinereum related to the pars tuberalis or the primary plexus On naked eye examination of the base of the brain the presence of the pars tuberalis and the primary plexus gives to the median eminence a pink colour in contrast to the grey or white colour of the adjacent regions of the tuber cinereum and hypothalamus The blood from the primary plexus is

drained down the large portal trunks which lie mainly on the anterior or ventral surface of the pituitary stalk In most forms such as the rat and man these vessels are clearly visible to the naked eye The portal trunks in turn break up and distribute their blood into the sinusoids of the pars distalis of the adeno hypophysis It is an interesting feature that the capillary loops

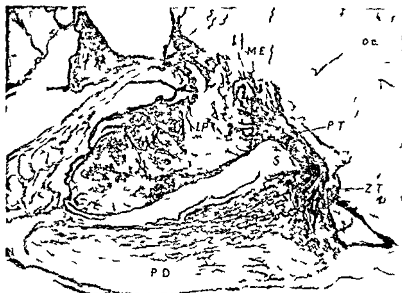


Fig 18 Photomicrograph of a midline sagittal section through the hypothalamus and pituitary gland of a porpoise
Blood vessels injected with indian ink Note the pars tuberalis *P T* rises anterior to the septum *S* to encircle the tuber cinereum and that the mass of sinusoidal capillaries in the median eminence *M E* of the tuber cinereum connect with the trunks of the portal vessels in the pars and zona tuberalis *Z T* and so with the sinusoids in the pars distalis *P D* *I P* infundibular process *O C* optic chiasma × 13
(From Harris G W (1950) *J Physiol* 111 361)

seen in the primary plexus of most higher mammals are probably representative of a primitive type of cerebral vascularization Many of the lower vertebrates as well as some marsupials (opossum) are described as having only non anastomosing vessels with terminal capillary loops in the brain and spinal cord The looped vessels illustrated in the median eminence of the rabbit

(Fig 14) and the dog (Fig 17) are very similar to the vessels shown by Horne Craigie (1938 Figs 2 3 and 4) and others in the central nervous systems of cyclostomes urodeles and reptiles

The exact pattern of the portal vessels varies between different species and seems to be largely dependent on the length and



Fig 19 Transverse section through the hypothalamus and pituitary gland of a rhesus monkey

Blood vessels injected with India ink Note the tufted vessels in the median eminence (M E) passing inferiorly as the portal vessels (P V) into the pars distalis Hypothalamus *H* × 12

(From Wislocki G B (1938) *Res Publ Ass nerv ment Dis* 17 48)

direction of the pituitary stalk (Green and Harris 1947 Harris 1950 Green 1951*a*) In the rat the primary plexus is most obvious as a midline tuft of vessels passing upwards into the median eminence (Fig 10) The pituitary stalk in the rat is a relatively long structure which passes almost horizontally backwards and the trunks of the portal vessels are similarly long and placed in the same plane (Fig 12) It is thus possible for a single

horizontal section (as in Fig 11) to pass through the tuber cinereum pituitary stalk and pars distalis In forms like the dog and cat in which the pituitary stalk is very short the trunks of the portal vessels are likewise short In this case the primary plexus is closely approximated to the sinusoids of the pars distalis so that the blood passes directly from the upper capillaries (primary plexus) into the lower capillaries (sinusoids of the pars distalis) without traversing any intervening portal trunks (Figs 16 17) The vessels of the rabbit are intermediate between those of the above extreme types (rat and dog) In this animal the pituitary stalk is abbreviated but trunks of the portal vessels do exist in a specialized part of the pars distalis known as the zona tuberalis The richness of the primary plexus in the median eminence of the rabbit is well shown in Fig 14 and the trunks of the portal vessels descending in the zona tuberalis before merging with the vessels in the pars distalis generally are shown in Fig 15 The portal vessels of the Cetacea are of note in that a septum intervenes between the anterior and posterior lobes of the pituitary as in the armadillo Indian elephant and other forms and it became of interest at one time to see whether the septum interrupts this vascular path A study of the hypothalamus and pituitary gland of the porpoise showed that the pars and zona tuberalis pass upwards anterior to the septum and that the portal vessels are present and more obvious than in some other forms owing to the fact that they are concentrated together (see Fig 18) The portal vessels of the monkey in which the pituitary stalk runs in a vertical plane are well described by

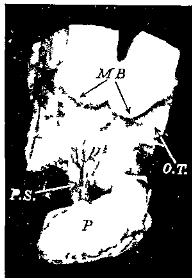


Fig 20 Photograph of hypothalamus stalk and pituitary gland of a man Posterior view
MB mammillary bodies *O T* optic tract *P* pituitary gland *P S* pituitary stalk posterior aspect showing portal vessels engorged with blood × 4 (From Green J D & Harris G W (1947) *J Endocrinol* 5 136)

Wislocki (1938) and illustrated in Figs 9 and 19 In man the pituitary stalk is long and runs downwards and forwards instead of downwards and backwards as in lower mammals The primary plexus in man differs from that of other forms in several ways (Green 1948 Xuereb Prichard and Daniel 1954) Firstly it is situated not only in the median eminence but also extends well down the neural stalk Secondly the capillaries that form the primary plexus are arranged in a curious tufted fashion as in the

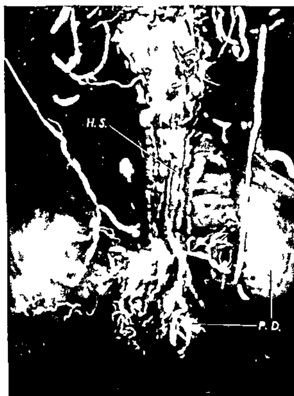


Fig 21 Photograph of anterior a pect of p tuitary stalk of man
Blood vessels injected with neoprene latex Note prom nent trunks of portal vessels in hypophysial stalk (*H S*) runn ng down into pars distal s (*P D*) wh ch s partly dissected to show the vessels breaking up nto the sinusoids of the gland × 10
(From Xuereb G P Prichard Marjorie M L and Daniel P M (1954) *Qua t J exp Physiol* 39 219)

monkey, and thirdly there appears to be more connective tissue (collagen and reticular fibres) in the walls of the tufted capillaries However in principle, the arrangement is the same in that blood passes from these wide capillaries situated in the neural tissue into large portal trunks which sweep on to the anterior surface of the lower end of the hypophysial stalk and so down into the pars distalis (Figs 20 21)

In all forms it seems that the capillaries of the primary plexus are situated in intimate relationship with nerve fibres in the median eminence, but they are far removed from any compact group of cells such as the supraoptic nucleus paraventricular nucleus or other nuclear groups This point is of significance when considering the possible influence of the hypothalamus on these vessels

(iii) *The direction of blood flow in the hypophysial portal vessels* For many years after the original observations of Popa and Fielding and Wislocki the direction of blood flow in the portal vessels was debated at times quite vehemently It is now possible *to state dogmatically that the direction of flow is from the median eminence to the pituitary gland* The evidence on which this statement is based has been summarized as follows (Green and Harris 1949)

(*a*) The anatomical arrangement of the vessels as seen on microscopic examination Arteries supply the pars tuberalis plexus and therefore the primary plexus but the only route for draining blood from these plexuses appears to be the portal vessels

(*b*) Observations made on glands in which the vessels had been incompletely filled after perfusion with indian ink

(*c*) The site of arrest of fat emboli within the system

(*d*) Slow vascular injection of indian ink in dead animals with observation of the filling of the portal vessels

(*e*) Indian ink injections made into the aorta of anaesthetized rats with intact hemisected or transected pituitary stalks followed by immediate decapitation

(*f*) Direct microscopic observation of the direction of blood flow in the portal vessels of anaesthetized animals

Clearly the last type of evidence is the most satisfactory and such observations have been made on amphibians by Houssay Bia

sotti and Sammartino (1935) and Green (1947) and on rats by Green and Harris (1949) In the anaesthetized rat it is possible to remove the lower jaw and part of the base of the skull and to observe the whole extent of the portal vessels microscopically With such a method it was observed that the blood stream in the portal trunks shows no sign of pulsation indicating its previous passage through a plexus and that the flow of blood is from the dense capillary plexus in the median eminence to the pars distalis of the pituitary gland There can be little doubt from this and the other evidence that a similar direction of flow will be found in other forms

(iv) *Embryological development of the pituitary gland and portal vessels* The embryology of the pituitary gland has been studied in many types of vertebrates Typical of these accounts is that given by Atwell (1918) for the rabbit (Fig 22) As is well known the adenohypophysis is derived from Rathke s pouch which is evaginated from the ectoderm of the stomodaeum whereas the neurohypophysis is derived from the floor of the diencephalon By the eleventh day of embryonic life in the rabbit the duration of pregnancy being about thirty two days in this form Rathke s pouch is well formed and two small protuberances at the rostral and lateral corners of the pouch denote the formation of the lateral lobes By the sixteenth day of pregnancy the stalk of Rathke s pouch has separated from the buccal ectoderm and the two lateral lobes have grown upwards to establish contact with the tuber cinereum By the twentieth day the lateral lobes are extending nasally and caudally to form the collar like plate of cells the pars tuberalis which finally encircles the tuber cinereum According to Dawson (1937) the lateral lobes of Rathke s pouch give rise not only to the pars tuberalis of the adenohypophysis but also by fusing in the midline to the anterior pole of the pars distalis known as the zona tuberalis This region of the pars distalis may be distinguished from the main mass of the pars distalis by its cytology (see Dawson 1948) and by its relationship to the trunks of the portal vessels In many forms it is partially separated from the more caudal part of the pars distalis by connective tissue trabeculae which appear to be incorporated in the gland by the separate upgrowth of the lateral lobes of Rathke s pouch in the embryo A systemic arterial supply makes its entry into the gland (at least in the rabbit and human) along the connective

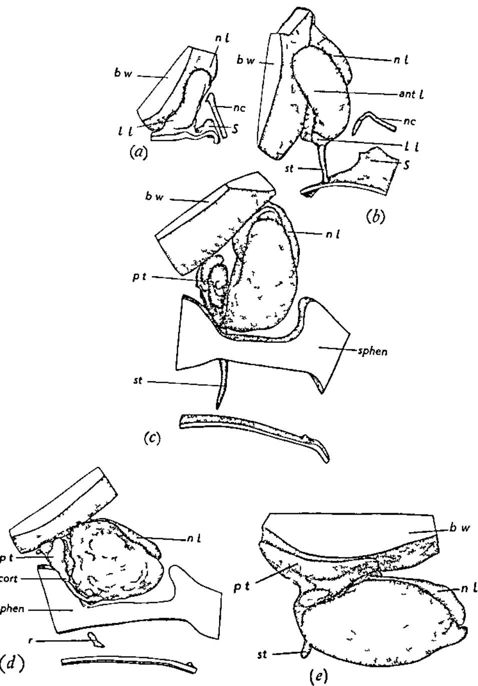

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Fig 22

tissue trabeculae whereas the portal vessels derived from the region of the pars tuberalis pass inferiorly in a concentrated mass in the zona tuberalis and therefore lie anterior to the trabeculae This relationship is shown in Fig 23 It seems then that the

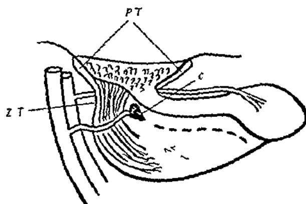


Fig 23 Diagram to show the relationship of the systemic arterial and portal vessel supply to the pituitary gland of a rabbit

C connective tissue trabeculum incorporated in the adenohypophysis by the upgrowth of the lateral lobes of Rathke s pouch These lobes form the zona (*Z T*) and pars tuberalis (*P T*) of the adult gland with which parts the hypophysial portal vessels are intimately related

lateral lobes of Rathke s pouch which in all probability form the pars and zona tuberalis are responsible also for the gross architecture of the blood vessels of the anterior lobe

Since the method of development of the pars tuberalis from the lateral lobes of Rathke s pouch is constant over a wide range of vertebrates the question arises as to the functional significance

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Fig 22 To show the embryological development of the pituitary gland of the rabbit

Note the upgrowth of the lateral lobes of Rathke s pouch which ultimately incorporate connective tissue trabeculae in the gland and form the pars and zona tuberalis of the adenohypophysis Compare the development of the different parts of the gland with the arrangement of the vascular system as shown in Fig 23

(*a*) 1 th day × 60 (*b*) 15th day × 60 (*c*) 16th day × 60 (*d*) 18th day × 38 and (*e*) 24th day of foetal life × 33

ant l anterior lobe *b w* brain wall *co t* cortical plate *l l* lateral lobe *nc* notochord *n l* neural lobe *p t* pars tuberalis *r* remains of stalk of Rathke pouch below sphenoid *S* Seessel pouch *sphen* portion of cartilage of sphenoid *st* stalk of Rathke s pouch

(From Atwell W J (1918) *Amer J Anat* 24 271)

of the definitive structure (pars tuberalis ± zona tuberalis) Since the pars tuberalis (1) has no proven endocrine function (2) constantly establishes contact with the floor of the diencephalon even in those forms in which a septum separates the pars distalis from the neurohypophysis and (3) in nearly all forms examined bears a constant relationship with the portal vessels it has been suggested (Harris 1947) that this part of the pituitary is laid down in the embryo as a bed for the hypophysial portal vessels In other words that the pars tuberalis may not be composed of endocrine tissue but is formed as the anatomical bed of a vascular pathway which constitutes part of an essential link between the central nervous system and the pars distalis of the pituitary There is some evidence against this view In a few exceptional forms such as the frog, the pars tuberalis becomes detached from the pars distalis and exists as two discrete patches of cells on the ventral surface of the infundibulum (Green 1947) Wingstrand (1951) has observed also in bird embryos that the portal vessels and pars tuberalis are formed separately and that as a secondary process the cells of the pars tuberalis formed from the two lateral lobes enclose the midline portal vessels from both sides However whether the teleological viewpoint is adopted or not it is clear that there is some close relationship between the pars and zona tuberalis and the portal system

(v) *Phylogenetic development of the hypophysial portal vessels* The following account of the evolutionary development of the portal vessels follows the description given by Green (1951*a*) (see Fig 24) Green found that in cyclostomes and fishes a plexus of vessels is present between the neurohypophysis and adenohypophysis In some forms this plexus penetrates the neurohypophysial tissue but in others it is a simple superficial plexus which probably nourishes the nervous tissue by diffusion though branches pass from it to supply the adenohypophysis In fishes this plexus may be greatly folded thus increasing its surface area If humoral transmission occurs from the neurohypophysis to the adenohypophysis the presence of this plexus and branches of supply to the adenohypophysis would probably make the transmission more effective In the Urodela (amphibians) a typical neural lobe with an independent blood supply appears for the first time and the plexus of vessels described above penetrates a specialized median eminence before supplying the pars

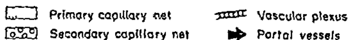


Fig 24 Phylogenetic development of the hypophysial portal vessels in the vertebrates

A H adenohypophysis *M E* median eminence *N H* neurohypophysis *N L* neural lobe *O C* optic chiasma *P D* pars distalis *P I* pars intermedia *P Te* pars terminalis *S V* saccus vasculosus *V L* ventral lobe Arrows indicate direction of blood flow Heavy arrows portal vessels

(From Green J D (1951) *Amer J Anat* 88 225)

distalis In the Anura (such as frogs and toads) the plexus not only penetrates the median eminence but collects into trunks destined to supply the pars distalis In the higher vertebrates (reptiles birds and mammals) a system of portal vessels passing from the median eminence to the pars distalis is constantly present Green (1951*a* page 274) concludes *It is a remarkable* fact that the hypophysio portal circulation shows such minor variations between related species and that the variations de scribed can be followed with such ease in so orderly a manner in a phylogenetic series Such constancy suggests a functional signific ance Were it not so wide variations might be expected to occur since the general morphology of the pituitary itself is anything but constant

(vi) *Regenerative capacity of the hypophysial portal vessels* It has recently been found that the hypophysial portal vessels possess marked powers of regeneration after section of the pituit ary stalk Following this operation in the rat it was found (Harris 1950*b* Harris and Jacobsohn 1950) that capillary outgrowths bridge the gap of the divided stalk as early as 24–48 hours after operation and that relatively large vascular trunks may cross the site of section within a few weeks The extraordinary capacity of these vessels to regenerate is shown by the fact that a capillary network may penetrate the interstices of a plug of cotton wool or gun cotton placed between the divided ends of the pituitary stalk and in this way may re establish vascular connection be tween the median eminence and anterior pituitary (rat—Harris 1950*b* rabbit—Brown Grant Harris and Reichlin unpublished) In the same studies on the rat and rabbit it was found that similar regeneration may occur around the borders of waxed paper plates placed between the cut ends of the stalk Harris and Jacobsohn (1952) reported that a profuse outgrowth of vessels may occur from the primary plexus into homotransplants of pituitary tissue placed in the subarachnoid space below the median eminence of the rat Extension of this work in the rabbit has given confirmatory results (Jacobsohn, 1954) Similar regener ation following pituitary stalk section, has now been observed in the mouse (de Groot 1952) ferret (Thomson and Zuckerman 1953 1954 Donovan and Harris 1954) and monkey (Harris and Johnson 1950) The possible significance of these findings in relation to experiments involving stalk section is apparent

(vii) *Functional significance of the hypophysial portal vessels* In the last few years much evidence has accumulated that the presence of a hypophysial portal circulation is essential for functional activity of the anterior lobe of the pituitary gland and that nervous (hypothalamic) factors may regulate the rate of secretion of the gland via this vascular system It is felt that the most likely hypothesis is that nerve fibres from the hypothalamus liberate some humoral substance(s) into the capillaries of the primary plexus in the median eminence and that this substance is carried by the portal vessels to excite or inhibit the secretion of the gland cells in the pars distalis This possibility is discussed in more detail in Chapter 7

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CHAPTER 3

TRANSPLANTATION OF ENDOCRINE GLANDS

The clearest single piece of evidence that a particular organ is an endocrine gland is that the organ will still maintain its normal function if transplanted to a distant site in the body Berthold (1849) gave the first proof of the existence of an internal secretion by transplanting the testes in castrated cocks and demonstrating that such transplants would maintain the normal male development of the bird The successful transplantation of a gland in this manner demonstrates two facts that the stimulus to the normal activity of the gland is mediated via the blood stream and that the gland produces its characteristic effects in the body via a secretion passing into the blood stream

The same general principles apply to transplantation of endocrine glands as of other tissues of the body (see review by Loeb 1930) Autografts (grafts in the same individual) readily take and survive homografts (grafts from another individual of the same species) may take and survive for a period though they often undergo lymphocytic infiltration and fibrosis after a period of some weeks and heterografts (grafts from an animal of another species) rarely take at all Take in this connection is used to denote the process by which a graft acquires a blood supply and remains histologically differentiated though it may or may not show signs of functional activity Most endocrine transplants will take more readily if there exists a state of deficiency in the host for the particular gland concerned This is known as Halsted s Principle of Deficiency and probably indicates that the glands for which the principle holds true are under the humoral control of one of the anterior pituitary trophic hormones For example ovariectomy is known to increase the secretion of gonatrophic hormones by the anterior pituitary so that ovarian grafts in an ovariectomized animal might be expected to take more readily than in the normal animal Similar factors probably operate in grafts where the testes thyroid and adrenal cortex are concerned There is however no evidence that a state of defi-

ciency is an aid to successful transplantation of the parathyroids adenohypophysis neurohypophysis or adrenal medulla

When studying the replacement capacity of an endocrine transplant in an animal in which the gland under consideration has been removed attention should be paid to the following points (Harris 1948)

1 The transplant should be situated at a distance from the normal site of the gland to obviate the danger of local vascular or nervous repair
2 The study should be extended over a period of time sufficient to ensure that any effects observed are due to functional activity of the transplant and not solely to the absorption of an implant
3 Serial sections should be made through the normal site of the gland and through the site of the transplant so that histological study may control the activity of the transplant and the complete absence of the animal s own gland The presence of accessory nodules of glandular tissue should also be excluded

Neurohypophysis and adrenal medulla

At least two endocrine glands have not been successfully transplanted the neurohypophysis and adrenal medulla Both these glands are characterized by their embryonic derivation from the neural ectoderm by being richly innervated and by the relatively short latency of the responses produced by their secretory activity It seems likely that their innervation and their acute short lived responses are to be correlated The more slowly acting endocrine glands such as the gonads and thyroid, are maintained and regulated humorally by their blood supply whereas these more quickly acting glands are dependent on a more quickly acting control mechanism

The degree of dependency of the neurohypophysis and adrenal medulla on a nerve supply is very marked If the hypothalamo hypophysial tract is interrupted by section of the pituitary stalk or by a hypothalamic lesion the neurohypophysis ceases to function and atrophies Similarly cutting the splanchnic nerves results in reduced function of the adrenal medulla which becomes relatively inexcitable to electrical stimulation (Cannon and

Rosenblueth 1937) It is hardly surprising therefore that transplants of these glands although they may remain recognizable to histological examination show relatively little if any function This fact is almost certainly related to their mode of control and not to their mode of action

Ovaries

Since the classical work of Knauer (1896) who grafted pieces of ovaries into ovariectomized guinea pigs and showed that such a procedure prevented castrate atrophy the replacement capacity of ovarian transplants has been confirmed many times Marshall and Jolly (1907 1908) were the first to make a detailed study of such transplants and to publish illustrations of microscopic sections of transplanted ovaries Autografts and homografts in inbred strains of animals give a high incidence of successful transplants and there is evidence that the number of successes is increased by previously removing the gonads of the host In successful cases changes typical of normal ovarian cycles occur that is follicular ripening ovulation and formation of corpora lutea The occurrence of atretic follicles and cystic follicles seems however to be more common than in normally situated ovaries

The fact that transplanted ovaries in an adult female host are capable of maintaining the accessory reproductive organs in a normal state indicates that the transplant is both controlled by and exerts its influence through humoral channels If however the ovary is transplanted to a site drained by the intestinal portal vein for example into the spleen then somewhat different results are obtained from those given above The transplanted gland may still undergo the cyclical changes but its effect on the accessory reproductive organs is reduced This is seen more clearly in lower mammals such as rodents and there is good evidence that the apparent reduction in ovarian activity is due to the fact that the steroid hormones are metabolized in the liver and do not therefore reach the uterus and vagina through the general systemic circulation

It has been known since the time of Foa (1900) that the ovary of an immature animal grafted into an adult host undergoes rapid development and assumes adult function long before it would normally have done so Also ovaries from adult animals grafted into immature ho ts lose their functional activity and

ciency is an aid to successful transplantation of the parathyroids adenohypophysis neurohypophysis or adrenal medulla

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between two rats of the same inbred strain may give functional transplants (Ingle, Higgins and Nilson 1938). When the whole adrenal gland is transplanted the adrenal medulla as well as the adrenal cortex may persist and be found present by microscopic examination of the graft at a later date. Following the observation of Evans (1936) that enucleation of the adrenal may be followed by regeneration of cortical tissue from the remaining capsule, it was shown that transplants of the cortex may be obtained by grafting pieces of the capsule into various sites (Turner, 1939; Baker and Baillif 1939).

Thyroid

Transplants of the thyroid gland have been studied by many workers (Manley and Marine 1916, Loeb, 1918, 1930, Marine and Rosen 1934, and others). Autografts of thyroid tissue take readily in many parts of the body; homografts often take but usually stimulate an immunological reaction and are absorbed during the first few weeks after transplantation, whilst heterografts are never successful. The growth of transplanted thyroid tissue is stimulated by a state of thyroid insufficiency in the host whilst administration of thyroid extract inhibits the development of a graft. It is likely that both these factors, thyroid insufficiency and thyroid extract administration, influence the growth of transplanted thyroid tissue indirectly, by affecting the rate of release of thyrotrophic hormone (T.S.H.) from the pituitary gland. Marine and Rosen (1934) showed that transplanted thyroid tissue reacted to thyrotrophic hormone probably to the same degree as the normal thyroid, and Bondy (1951) and Bennett and Gorbman (1951) have produced evidence of normal thyroid function after transplantation.

Parathyroid

Successful autotransplants of the parathyroid glands have been obtained (Leischner 1907, Halsted, 1909, Manley and Marine, 1916) and cases of successful homotransplants have been recorded in human patients suffering from tetany (Manfredi and Castillo 1941, Stone, Owings and Gey, 1934). Manfredi and Castillo used the parathyroid glands of new born infants for grafting since the possibility of agglutination reactions with foetal blood is slight. Stone, Owings and Gey cultured the parathyroid tissue

become quiescent These old observations that have been amply confirmed, indicate that the activation of the ovary at puberty is not due solely to an increased sensitivity of ovarian tissue to gonadotrophic hormones, but also to an increased blood concentration of these hormones From this evidence it seemed possible that the onset of puberty was dependent on ageing and maturation of the anterior pituitary gland but it now seems likely that other factors are involved (see Chapter 4)

Most workers have found that ovaries transplanted into castrate male hosts may become active as shown by the develop ment of ripe follicles but that ovulation and formation of corpora lutea does not occur (Goodman 1934 Deanesly 1938) The ripe follicles in such transplants however, will undergo luteinization if the host animal is injected with pituitary extract It has been argued from these experiments that anterior pituitary tissue of male animals does not release follicle stimulating hormone (F S H) and luteinizing hormone (L H) in the same rhythmic fashion as the female gland and that this might be due to some sex difference in anterior pituitary tissue (See however, p 95)

Testes

Functional autografts and homografts of the testes have been obtained by many workers (review by Moore 1939) In warm blooded animals it is easier to obtain grafts showing maintenance of interstitial tissue than grafts showing sperm formation This is probably due to the temperature of the site chosen for the transplant for Moore (1926) found that testes grafts made into the scrotum of guinea pigs and rats showed active spermatogenesis six months later and Turner (1938) obtained similar results in rats when testes were transplanted from new-born animals into the anterior chamber of the eye of the hosts Both the scrotum and the anterior chamber of the eye are several degrees cooler than the general body temperature and this fact may well account for the spermatogenesis seen in these cases

Adrenal cortex

Transplants of the adrenal cortex take and grow readily pro viding there is a state of adrenal cortical deficiency in the host (Wyman and Tum Suden 1937 and others) Homotransplants are less successful than autotransplants though cross grafting

after killing the grafted animal Examination of the sella turcica under a dissecting microscope is insufficient

One of the earliest attempts to transplant pituitary tissue was made by Crowe Cushing and Homans (1909) They made auto transplants into the rectus muscle bone marrow and cerebral cortex of dogs but had no clear criteria with which to judge any activity of their transplants Hohlweg and Junkman (1932) Gardner and Hill (1935) Martins (1936) Deschn and Gregoire (1936) Buxton (1936) and Emery (1936) confirmed Crowe Cushing and Homans (1909) that transplanted anterior pituitary tissue could obtain a blood supply and remain histologically identifiable but added little data regarding the functional ability of such transplants Hill and Gardner (1936) described two hypophysectomized male mice in which intratesticular grafts of pituitary tissue from litter mates were made It was found that the seminiferous tubules seminal vesicles and adrenal cortex were well maintained but serial sections through the pituitary region showed that both animals were incompletely hypophysectomized May (1935 1937) grafted pituitary tissue into the anterior chambers of the eyes of four rats (two male and two female) The testes of the male rats were maintained in an active state and the female rats regained oestrous cycles and became pregnant Hypophysectomy was said to be verified histologically but the fact that one female rat still had oestrous cycles after removing the eye containing the graft throws some doubt on this observation Richter and Eckert (1937) transplanted pituitary tissue into hypophysectomized female rats but did not observe oestrous cycles in these animals and the ovaries and adrenals were found to be atrophic Phelps Ellison and Burch (1939) made pituitary grafts into the thigh muscles of hypophysectomized female rats Some of these grafts survived but showed comparatively little functional activity Evidence of mild follicular stimulation was observed but no ovulation formation of corpora lutea or oestrous cycles were seen Ovarian and adrenal weights and histology also indicated some but comparatively little function of the transplants The completeness of hypophysectomy was determined by careful check at autopsy In a thorough study Westman and Jacobsohn (1940) transplanted the pituitary glands of young rats into the anterior chamber of the eye of hypophysectomized female rats The grafted animals were observed for periods up

in a medium containing the patient's serum, in an attempt to adapt the tissue to the blood composition of the future host before the operation of grafting was performed There is little evidence that the anterior pituitary gland has any controlling influence over the parathyroid, and it is of interest in this respect that a state of parathyroid deficiency is not essential to successful parathyroid grafting Manley and Marine (1916) observed many cases of successful parathyroid transplants in rabbits when some of the hosts own parathyroids were still in place

Anterior pituitary gland

In respect to transplantation the anterior pituitary gland differs markedly from the other endocrine glands discussed above The functional activity of the adenohypophysis is very markedly reduced if it is transplanted to a site in the body remote from the sella turcica Since the gland has at most a very scanty innervation it is improbable that this result is due to section of a *secreto motor nerve supply, as seems to be the case for the* neurohypophysis or adrenal medulla In lacking a well marked secreto motor nerve supply the anterior pituitary lies in the same category as the ovary testes thyroid and adrenal cortex and it might be thought from this that the mechanism regulating its activity and by which its function is stimulated under various conditions is likely to be of a humoral nature The fact that transplantation to a distant site does not result in a functionally active graft would indicate that this humoral mechanism is not the general systemic circulation

Since the statement that anterior pituitary tissue trans planted to a distant site shows very little functional activity is of such significance in the present discussion some of the older work which claimed that functionally active transplants had been obtained will be examined in more detail In evaluating work of this type the three criteria to which attention was drawn on page 42 should be born in mind It is especially important that the original hypophysectomy should be rigidly controlled if claims are made that transplanted pituitary tissue is functionally active *During the operation of hypophysectomy it is difficult to be cer* tain that a fragment of tissue has not been left *in situ* and this technical difficulty necessitates the control procedure of making serial sections through the whole extent of the pituitary capsule

was no evidence of extra stimulation of the adrenals under the stress of starvation Schweizer and Long (1950*b*) found some evidence of thyrotrophic activity maintained by such pituitary grafts in a few of their animals but at a greatly reduced level More recent work by Greer Scow and Grobstein (1953) has also

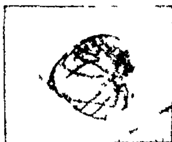


Fig 25 Photograph of the eye of a rat bear ng a transplant of anterior p tuitary tissue in the anterior chamber Note the large essels of the transplant visible through the cornea

(From Westman A and Jacobsohn Do a (1940) *Acta path microbiol sca d* **17** 328)



Fig 26 Microphotograph of a section through the iris of the eye of a rat with an attached anterior pituitary transplant

(From Westman A and Jacobsohn Dora (1940) *Acta path microbiol scand* **17** 328)

to five months before being killed After death the volume of transplanted tissue in the eye was measured and found to represent 30–200 per cent the normal volume of the rat s pituitary gland (Figs 25 26) These transplants were found to be well vascularized but contained few or no eosinophilic cells Their secretory activity was thought to be nil for gonadotrophic hormone doubtful for the growth hormone and slight but definite for the adrenocorticotrophic hormone Cutuly (1941) made grafts into the anterior chamber of the eye of hypophysectomized male rats One animal had a fertile mating 200 days after operation and in general there seemed to be some maintenance of testicular weight though no maintenance of the thyroid or adrenal glands The work of Schweizer and his colleagues on pituitary transplants into the anterior chamber of the eye of hypophysectomized guinea pigs is much quoted in the literature but their results in respect to the functional ability of their grafts are far from clear Schweizer Charipper and Haterius (1937) found such grafts in female guinea pigs might produce some follicular growth in the ovaries (which was not reflected by an increase in ovarian weight) hypertrophied uteri and constant vaginal opening (this latter occurring in two out of ten animals) No evidence of ovulation or formation of corpora lutea was seen although this normally occurs spontaneously in the guinea pig All the thyroids and adrenals examined showed good maintenance Schweizer Charipper and Kleinberg (1940) found that similar grafts in male guinea pigs could maintain the reproductive organs in an active state though the testis weight was reduced as compared with the normal A puzzling fact in these experiments was the striking gain in body weight due to growth which occurred in control animals that had been hypophysectomized and implanted but in which the implants had not taken However the method of verifying the completeness of hypophysectomy—scraping the sella turcica and serially sectioning questionable fragments—is open to doubt In these experiments on male guinea pigs there was no evidence of any maintenance of the adrenal glands though in later work Schweizer and Long (1950*a*) produced histological evidence that partial maintenance of the adrenal gland could be obtained with intraocular pituitary transplants in male and female guinea pigs In no case however did the graft support the gland at a normal level of activity and there

by intraocular transplants in rats but no maintenance of body growth or of the testes These last three groups of workers (Cheng et al 1949 McDermott et al 1950 and Fortier 1951) all found evidence that the rate of A C T H secretion by the grafted tissue although below normal levels could be affected by various procedures such as administration of histamine or adrenaline This point will be referred to again later (Ch 5) for the moment it is sufficient to note that all agreed that there was no evidence for secretion of the growth or gonadotrophic hormones and that the adrenal glands of their transplanted animals were partially or completely atrophic

Haymaker and Anderson (1936) and Martinovitch (1950) tried a different technique in an attempt to obtain functionally active pituitary transplants These workers made cultures of pituitary tissue from young rats and after 5–7 days in the first case or 32 days in the latter, transplanted the cultured tissue to hypophysectomized recipients Haymaker and Anderson studied 89 animals and found some evidence for sperm production and thyroid function in 3 and some evidence of adrenal repair in 5 Martinovitch observed some resumption of growth in the five rats he examined

Summarizing the above results it may be said that there is no evidence that the anterior pituitary gland will maintain normal functions if transplanted to a distant site in the body and that in this respect the adenohypophysis stands in marked contrast to the ovaries testis adrenal cortex thyroids and parathyroids However very different results are obtained if the pituitary gland is removed from the sella turcica so that all vascular and nervous connections are interrupted, but is then replaced *in the sella turcica or in an adjacent site* Normal anterior pituitary function may then return to such grafted tissue To differentiate this procedure from that described above where the gland was replaced in a distant site the words grafting and grafts (instead of transplanting and transplants) will be used in this connection Greep in 1936 was the first to report that young (28 day old) hypophysectomized rats bearing auto and homo grafts of pituitary tissue in the sella turcica showed good anterior pituitary function as evidenced by growth oestrous cycles pregnancy and lactation One difficulty in the interpretation of these results was the impossibility of determining the completeness of the

demonstrated some maintenance of thyroid function by intraocular pituitary transplants in hypophysectomized mice These workers found that the transplants did not maintain the body weight or the weights of the thyroid ovaries adrenals or uterus significantly above those of hypophysectomized controls However thyroid function as measured by a radioiodine uptake per unit thyroid weight and a thyroid/serum iodide ratio was apparently maintained at about two thirds the normal level From these and other results Greer (1952) suggests there are two types of *thyroid stimulating hormone released from the pituitary the* first concerned with thyroid growth the secretion of which is controlled by the hypothalamus and the second concerned with thyroid activity the secretion of which is independent of the hypothalamus Further data are required for the substantiation of this view A study of the luteotrophic function of pituitary autografts has been made by Everett (1954) Adult female rats were hypophysectomized on the day following ovulation and the pituitary tissue transplanted to the renal capsule or the vicinity of the common carotid artery The uteri of these animals were traumatized four days later and examination at autopsy on the eighth day *revealed the development of deciduomata indicating* the maintenance of functioning corpora lutea over this interval Everett discussed the possibility that these results may be explained by the absorption of hormone from an implant rather than by functioning of a transplant *but felt this to be unlikely*

Since the clinical importance of the adrenocorticotrophic hormone (A C T H) has been established several groups of workers have studied the activity of pituitary transplants with special reference to the mechanism controlling release of A C T H Cheng Sayers Goodman and Swinyard (1949) studied pituitary transplants in hypophysectomized rats and found The decrease in weight of the adrenals was in most instances as great as that which occurred in the hypophysectomized animals without grafts There was also little evidence of secretion of growth hormone or gonadotrophic hormone by the transplanted pituitary tissue McDermott Fry Brobeck and Long (1950) found intraocular transplants of anterior pituitary tissue would give partial maintenance of adrenal gland weights in rats but their animals did not grow and the testes were completely atrophic Fortier (1951) likewise observed some maintenance of the adrenal glands

by intraocular transplants in rats but no maintenance of body growth or of the testes These last three groups of workers (Cheng et al 1949 McDermott et al 1950 and Fortier 1951) all found evidence that the rate of A C T H secretion by the grafted tissue although below normal levels could be affected by various procedures such as administration of histamine or adrenaline This point will be referred to again later (Ch 5) for the moment it is sufficient to note that all agreed that there was no evidence for secretion of the growth or gonadotrophic hormones and that the adrenal glands of their transplanted animals were partially or completely atrophic

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initial hypophysectomy by serially sectioning the sella turcica This experiment does not seem to have been repeated in detail, although Cutuly (1941) found sellar grafts were more active in giving partial maintenance of the adrenal glands than intraocular transplants In the fifteen years succeeding Greep s observations evidence accumulated that the functional activity of the anterior pituitary gland is dependent on its blood supply via the hypo physial portal vessels If anterior pituitary activity and its neural regulation is dependent on these vessels (and not on a direct nerve supply or on humoral stimuli arriving via the general sys temic circulation) then one possible explanation of Greep s results seems to be that the portal vessels regenerated and re established the anatomical path from the hypothalamic region to the grafted pituitary tissue

In order to test this hypothesis Harris and Jacobsohn (1950 1952) made the following experiments Adult hypophysectom ized female rats were grafted with pituitary tissue taken from their own new born young (1–10 days old) since such new born tissue had been found to give good takes in a previous investiga tion (Westman and Jacobsohn 1940) The maternal rats were first hypophysectomized by the parapharyngeal route and then in a one stage operation the pituitary tissue was inserted by a temporal approach either in the subarachnoid space under the cut pituitary stalk or a few millimetres laterally under the tem poral lobe of the brain The advantage of placing the grafts in the subarachnoid space under the cut pituitary stalk instead of in the sella turcica is that the grafts are in a position where they may become revascularized by the hypophysial portal vessels but in a position also that enables the completeness of hypophy sectomy to be verified by serially sectioning the pituitary capsule These animals were allowed to survive for periods varying from 1 to 8 months and it was observed that all twelve animals with grafts placed under the pituitary stalk regained a normal oestrous rhythm On placing with normal males six became pregnant and delivered living young after 22–23 days pregnancy Milk secretion in these animals also appeared normal though the milk ejection reflex was interrupted through lack of functioning posterior pituitary tissue However replacement therapy (sub cutaneous injection of oxytocin three times daily) enabled one of the grafted animals to rear her young After death it was



Fig 27



Fig 28

Microphotographs of sagittal sections through the hypothalamus pituitary graft and base of skull of a rat × 50

Fig 27 shows an unstained ection 100 μ th ck Note the vascular connections passing from the primary plexus (*P P*) of the hypophysial portal vessels to the graft (*A L*) Indian nk injected spec men

Fig 28 An adjacent section through the same specimen as illustrated in F g 27 To show the graft cons sting of anterior lobe t ssue (*A L*) *P P* primary plexus 10 μ thick Haematoxyl n eos n

(From Harris G W & Jacobsohn Dora (1952) *Proc Roy Soc B* 139 263)

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atrophy had occurred in the adrenal glands but in a few cases this did not appear quite so marked as in the hypophysectomized rats without grafts Microscopic examination of the temporal lobe grafts demonstrated the survival of as much pituitary tissue which was as well vascularized (Figs 29 30) as in the case of pituitary stalk grafts

These experiments then afforded study of two series of hypo physectomized adult female rats bearing grafts of pituitary tissue obtained from their own young In both groups the grafts were placed in the subarachnoid space and in both the anterior pituitary tissue took well and became well vascularized by cere bral vessels In one group the vessels of supply were mainly derived from the hypophysial portal vessels and in most of these animals anterior pituitary function so far as it was observed (gonadotrophic adrenocorticotrophic thyrotrophic and lacto genic) was normal whilst in the other group the vessels of supply to the grafts came from the blood vessels of the temporal lobe of the brain and there was very little if any anterior pituitary function to be discerned It is difficult to escape from the con clusion that the hypophysial portal blood supply has some specific effect in activating anterior pituitary tissue Other poss ible explanations of these results such as the existence of hypo thalamic secreto motor nerve fibres reinnervating the grafts are extremely unlikely—since fibres of this type cannot be found in normal glands and would almost certainly not regenerate after section if they were present [Such nerve fibres would come into the category of fibres belonging to the central nervous system and it is well known that these fibres in mammals have very little power of regeneration It has been shown that the nerve fibres of the pituitary stalk do not regenerate after section (Fisher Ingram and Ranson 1938 Magoun Fisher and Ranson 1939)] The results are best explained on the view that nerve fibres of the hypothalamus liberate some humoral substance into the primary plexus of the hypophysial portal vessels and that these vessels transmit the substance to the adenohypophysis where it exerts an activating effect on the gland cells This theory would explain why such glands as the gonads thyroid and adrenal cortex which receive their stimulus to normal activity (pituitary trophic hormones) via the general systemic circulation may be trans planted to distant sites in the body and still remain functionally

found that these animals possessed ovaries reproductive tracts adrenals and thyroids that were indistinguishable from those of normal control animals Serial sections through the grafts and surrounding structures demonstrated that the grafted tissue had acquired rich vascular connections with the hypophysial portal vessels in the median eminence (Figs 27 and 28) and that the



Fig 29



Fig 30

Microphotographs of sections through a pituitary graft placed under the temporal lobe of the brain of a rat

Fig 29 To show vascularity of the graft Indian ink injected specimen 100 μ thick unstained × 50

Fig 30 Adjacent section through the same specimen as illustrated in Fig 29 showing that the graft consists of well maintained pituitary tissue 10 μ thick section haemotoxylin eosin × 105

(From Harris C W & Jacobsohn Dora (1952) *Proc Roy Soc B* 139 263)

anterior lobes of the grafts contained well differentiated cells of all types though the neural lobes were atrophic The rats in which pituitary tissue was placed under the temporal lobe of the brain gave results which were strikingly different Oestrus was not observed in any of these animals and at post mortem the ovaries reproductive tract and thyroids were found to be as atrophic as those of hypophysectomized control rats Similar

activity is not due to any intrinsic property of the tissue itself but to some outside drive or stimulus derived probably from the central nervous system

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active whereas the anterior pituitary which normally is activated by its own local portal blood supply becomes inactive if transplanted to a distant site It is also tempting to speculate on the frequency with which metastasizing tumours of the testis ovary thyroid and adrenal cortex are observed clinically and the rarity of secondary metastases from tumours of the anterior pituitary gland (see King 1951)

The experiments on grafting pituitary tissue under the pituitary stalk (Harris and Jacobsohn 1952) also furnished some data of more general interest in pituitary physiology

1 The role played by the posterior pituitary gland in the process of milk ejection from the maternal mammary gland to suckling young—this is discussed in more detail in Chapter 10

2 The lack of sexual differentiation of anterior pituitary tissue In some further experiments the anterior pituitary of adult male rats were placed under the pituitary stalk of hypophysectomized female rats This male pituitary tissue was observed to support normal oestrous cycles and pregnancy in the female hosts indicating the secretion of F S H and L H after the female pattern Now ovarian tissue transplanted into castrate male rats has been found by most workers to develop ripe follicles which do not undergo ovulation or luteinization and this has been taken to indicate a lack of L H secretion by the male pituitary gland Thus it would appear that the secretory pattern of anterior pituitary tissue is dependent on some extrinsic stimulus This conclusion is also supported by

3 The maturation of anterior pituitary tissue The twelve post parturient rats in which grafts of pituitary tissue from their own young were placed under the pituitary stalk showed the recurrence of normal regular oestrous cycles at a time when their litters would have been 12–43 days old This demonstrates that immature pituitary tissue grafted into adult hosts shows hastened development, and in correlation with the fact that immature ovaries grafted into adult hosts also show hastened development, indicates that the onset of pituitary gonadotrophic ovarian activity at puberty depends on some factor other than maturition of pituitary and ovarian tissues This factor is possibly a stimulus emanating from the hypothalamus It may be argued from the results of these experiments that anterior pituitary tissue is plastic in nature and that its pattern of secretory

activity is not due to any intrinsic property of the tissue itself but to some outside drive or stimulus derived probably from the central nervous system

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CHAPTER 4

REGULATION OF GONADOTROPHIC SECRETION FROM THE ANTERIOR PITUITARY GLAND

The overall view of the mechanism to be dealt with in this chapter is shown diagrammatically in Fig 31 Changes in the external environment and psychological upsets may influence sexual processes and it is very likely that this is brought about via the central nervous system influencing the secretion of gonadotrophic hormones from the anterior pituitary gland As is well known the gonadotrophic hormones regulate the activity of the gonads (ovaries and testes) but do not have any direct influence on the accessory sexual organs (uterus vagina, seminal vesicles or prostate) with the possible exception of the mammary gland The gonadal hormones in turn regulate the accessory sexual organs These latter hormones by reacting back on the central nervous system (and possibly on the pituitary gland) via the general systemic circulation form a type of feedback mechanism which adjusts the animal s behaviour to conform with the hormone balance of the different phases of the sexual cycle and also adjusts to some extent the anterior pituitary output of gonadotrophins

Evidence for Neural Control of Gonadotrophic Secretion

Dr F H A Marshall of Cambridge was one of the first to emphasize the close correlation between the external environment and reproductive processes He drew attention to the fact that a variety of environmental factors such as food light temperature presence of a mate and so on are of importance in conditioning sexual periodicity It is likely that the intrinsic sexual rhythm of many mammals is adjusted to external seasonal changes by such exteroceptive factors as Marshall termed them For a detailed discussion of the part played by exteroceptive factors in reproduction the reviews of Marshall (1936 1942 1955) should be consulted

The majority of animals have a more or less definite season or

seasons of the year during which they breed Insectivores carni vores rodents and non ruminating ungulates tend to breed in the spring in both northern and southern hemispheres Rumin ating ungulates on the other hand tend to breed in the autumn If the environmental conditions under which an animal lives are reversed i e by transference across the Equator the time of the breeding season may also be reversed (Marshall 1937) This changeover in breeding season has been observed in birds

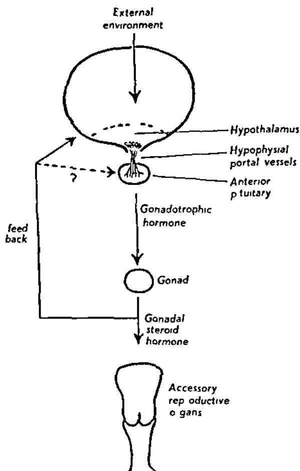


Fig 31 To illustrate the relationships between the external environment and the reproductive organs

ruminants and other mammals transported from the southern to the northern hemisphere and vice versa

Domesticity appears to confer some release of the breeding season from the seasons of the year The domestic bitch has usually two oestrous cycles in a year each one of six months duration These cycles occur rhythmically though independently of the seasons as opposed to those of the fox wolf or jackal which occur only in the spring Similarly the domestic cat may have two or three breeding seasons in the year whilst the wild cat confines its season to the spring Also the domestic rabbit if well cared for may breed at any time of the year whilst its wild counterpart breeds from February to May with a possible second season in the autumn Marshall remarks that this effect of domestication is perhaps partly the result of artificial selection but warmth good nutrition and comparatively uniform conditions are probably contributory causes Uniformity of the external environment as in some parts of the tropics where the climate and general conditions are fairly constant may also lead to breeding at any time of the year

The precise environmental factors which determine the breeding season appear to vary from species to species Food temperature humidity light the presence of eggs or young the presence of a companion or mate (apart from any ensuing pregnancy) and psychological changes dependent on the environment may all affect the sex rhythm in different forms The manner in which such factors produce their effects on the reproductive system has been summarized by Marshall (1942) as follows

it would appear certain that many external factors which regulate the cycle act through the intermediation of the central nervous system upon the anterior pituitary this gland playing the part of a liaison organ between the nervous system which is affected by stimuli from without and the endocrine system Some of these factors and evidence relating to the manner in which they produce their effects will now be discussed

Food

It has been known since the time of Aristotle that when food is abundant and other factors are favourable sheep may breed twice a year The process of flushing sheep consists of supplying them with extra food which is thought to hasten forward the

sexual season and to increase the crop of lambs Undernutrition if severe enough to cause marked weight loss may cause disturbance of the oestrous and menstrual cycles Inanition during war is often blamed for the amenorrhoea and menstrual irregularities that are seen at such a time but Bass (1947) who reported that amenorrhoea occurred in 54 per cent of women in a concentration camp stated that the loss of cycles occurred within four weeks of the beginning of internment which makes it unlikely that nutritional factors were involved Stroink (1947) reported similar cases during the occupation of Holland and concluded that the main cause was psychic Amenorrhoea is common in the disease anorexia nervosa and is regarded by some clinicians as a necessary concomitant for the diagnosis to be made The probable sequence of events is that some psychological disturbance results in reduced appetite and food intake which in turn depresses anterior pituitary function Many investigations have been made on the effects of general inanition and specific (vitamin) deficiencies on the oestrous cycle There is often little evidence however as to how the effects are produced That inanition may act peripherally is shown by the findings of Goldsmith and Nigrelli (1950) that lack of food renders the accessory genital organs of mice less sensitive to the steroid hormones though the work of Nelson Lyons and Evans (1953) indicates that vitamin deficient diets may result in dysfunction of both the pituitary and ovary The effects of underfeeding on rats is of interest in that malnutrition in this animal may produce a condition similar to that following hypophysectomy and therefore termed pseudohypophysectomy (Mulinos and Pomerantz 1940 1941*a* 1941*b*) Rinaldini (1950) found however that the various organs of the rat undergo different degrees of atrophy during starvation or hypophysectomy and has also noted (Rinaldini 1949 Pearse and Rinaldini 1950) that after a period of inanition the gonadotrophic content of the pituitary is about three times that of the pituitary of the normal rat Starvation in this animal then would appear to increase the rate of formation of gonadotrophin or depress the liberation of this hormone into the blood Under such circumstances the latter hypothesis would appear more likely It is of interest in this connection that the effect of an inadequate diet on the reproductive capacity of the rat may be compensated by extra lighting or conversely

that rats may breed as efficiently under conditions of shortened illumination provided that the diet is adequate (Alexander and Frazer 1952) Since there is good evidence that light affects the reproductive system via the mediation of the central nervous system and the pituitary gland this observation raises the possibility that the effects of some dietary variations act at the same level Further evidence for this view is the fact that the ovarian atrophy which follows starvation in female rats may be prevented by administration of threshold doses of anterior pituitary extract or pregnant mare s serum (Werner 1939)

An excellent review has recently appeared dealing with the relation of nutrition to endocrine reproductive functions (Meites 1953) The conclusion is drawn that the only type of mal nutrition which has conclusively been proven to impair the secretion of gonadotrophic hormones by the pituitary is under feeding or any state leading to inanition, including inadequacies of B vitamins

Temperature

The temperature and humidity of the environment seem to play a part in determining the sexual cycle in some forms Many amphibians will only breed if the temperature is above a certain level and then only after rain (Bragg 1940) In birds and mammals also there is evidence that the external temperature affects gonadal function For example Lamoreux (1943) found that although variations in light had little effect upon comb growth in cockerels a low environmental temperature effectively inhibited such growth The sex rhythm of the field mouse hedgehog rat, ground squirrel and other mammals appears sensitive to changes in temperature It is of interest that a low environmental temperature may affect gonadal activity in these forms in different ways The female rat has been found to exhibit lengthened oestrous cycles when maintained at a low temperature (Lee 1926 Bohanan 1939) and this probably indicates a state of lowered gonadal activity whereas Wells and Zalesky (1940) found that the normally limited period of testicular activity in the male ground squirrel could be greatly extended by keeping the animals in a constant low temperature

Light

The principal stimulus relating reproductive rhythm to the environmental seasons appears to be light Rowan in 1926 was the first to demonstrate that extra illumination during the hours of darkness would result in active gonads in the migratory junco finch By experiments carried out in the open in Alberta during winter where the temperature may fall to —50 F he found it possible by giving extra illumination to cause enlargement of the sex glands with sperm production at this time of the year instead of at the usual breeding season in April or May This work was extended to mammals by Baker and Ranson (1932) who worked on the field mouse and by Bissonnette (1932) who studied ferrets Since that time observations made under both natural and experimental conditions have demonstrated that variations in light form a potent stimulus to breeding phenomena in many types of vertebrates Amongst the common mammals the reproductive rhythm of the mouse rat ferret hedgehog cotton tailed rabbit cat raccoon mink goat sheep and others is sensitive to changes in light exposure whilst that of the adult cotton rat squirrel guinea pig and rabbit is not

The manner in which light affects the sexual processes is not clear The wavelength or intensity of the light the duration of daily exposure to light the day to day change and rate of change of these factors have all been considered (Hammond 1951) Some of these factors have been investigated in the ferret This animal is well suited for such experiments since it has a well marked breeding season extending from about the middle of March to July or August and since the oestrous state in the ferret can be easily recognized on account of the vulval swelling Marshall and Bowden (1934) investigated the effect of irradiation with light of different wavelengths on the oestrous cycle of the ferret They found that the recurrence of oestrus in the female ferret in winter is greatly accelerated by extra illumination with lights of wavelengths varying from λ 6500 to λ 3650 (red radiation to near ultra violet) Over this range intensity appears to be more important than wavelength Marshall (1940) also showed that if female ferrets were subjected to different degrees of intensity of light irradiation the acceleration of the oestrous cycle was in general correlated with the degree of intensity There were however exceptions to this Female ferrets subjected to

ultra violet irradiation usually remained on heat much later in the year than those submitted to visible irradiation An action of darkness as well as light is suggested by the work of Hammond (1951) who found that ferrets exposed to 14 hours constant light daily came into oestrus sooner than those exposed to 24 hours light daily The latter group were in fact little ahead of what would have been expected in daylight The studies of Hart (1951) in the ferret also indicate that the total daily quantity of light is not the controlling factor in accelerating oestrus during *the winter months but that a long light short dark ratio each* day produces the maximum acceleration It would seem that to induce an oestrous state in female ferrets in winter extra light is essential but not to the exclusion of darkness A light dark sequence is of importance In the ferret the proportion of light to darkness required to accelerate oestrus is of the order of two parts or more of light to one part darkness in each 24 hours The ferret may therefore be referred to as a long day breeding animal in contrast to the sheep which normally breeds from early October to late March (Hammond 1944) and is therefore a short day breeding animal Yeates (1949) showed that sheep could be brought into oestrus if subjected to a gradually *decreasing* duration of light each day and Hart (1950) demonstrated that the light dark ratio required to accelerate oestrus in the sheep was the reverse of that in the ferret that is one part of light to two parts or more of dark

It is fairly certain that the effect of light on the reproductive organs is mediated via the eyes and the anterior pituitary gland Hypophysectomized ferrets do not respond to extra illumination (Hill and Parkes 1933) and those in which the optic nerves have been severed show either an absence of oestrous cycles or cycles freed from seasonal or photic influence (Bissonnette 1938, Clark McKeown and Zuckerman 1939) Thomson (1951) investigated the components of the optic nerve concerned in the oestrous response of the ferret to extra light After studying *ferrets in which the optic nerve fibres only* had been cut or in which the central artery to the retina and the long and short ciliary vessels and nerves had been cut or in which all these structures had been sectioned he concluded that the gonadal response to added light depends on the integrity of the ganglion cell layer of the retina In a more extensive report on the effect

of extra light on blinded ferrets Thomson (1954) is in agreement with previous workers but his experimental data are unsatisfactory owing to the techniques employed to blind the animals The three methods used were (i) section of the optic nerve fibres inside the sheath of the optic nerve (ii) midline sagittal section through the optic chiasma and (iii) incision between the posterior margin of the optic chiasma and the anterior limit of the hypothalamus From the neuroanatomy of the visual pathway there is no reason to believe that animals subjected to the latter two procedures at least would be blind and since post mortem examination of the brains of these animals was not reported this work is unconvincing The more central connections of the optic path underlying this phenomenon in the ferret were studied by Clark McKeown and Zuckerman (1939) These workers made lesions in or extirpated various parts of the brain and found that the normal response to visual stimulation occurs in the absence of the superior colliculi when all retinal impulses to any part of the midbrain had been interrupted, and when retinal impulses to the dorsal nucleus of the lateral geniculate body and the visual cortex had been completely interrupted The conclusion was suggested that the visual response depends on impulses passing either to the ventral nucleus of the lateral geniculate body or to the subthalamus by way of the accessory optic tracts However the brains of these animals were later studied by Jefferson (1940) who could find no evidence of accessory optic tracts or of optic connections with the ventral nucleus of the lateral geniculate body in the ferret From time to time reports appear describing optic fibres terminating in the hypothalamus For example Frey (1951) has described a hypothalamic optic root in the dog and other forms but such an account requires confirmation in the ferret At the moment it seems likely that light act via optic nerve fibres which transmit a stimulus in some way to the hypothalamus and the pituitary gland but the details of this path are still doubtful

The presence of eggs, young, a companion of the same sex or a sterile mate

In many birds the initial stages of seasonal sexual activity seem to be dependent not only on light but on external stimuli received from other members of the community Most workers

believe that the presence of large numbers of birds in a flock has a stimulating effect upon the individual members Darling (1938) suggests that the visual and auditory display of the other members of a flock may be indispensable to the reproductive activity of individual birds The sex display of many birds probably affords another example of mutual stimulation Marshall (1936, 1942) has proposed the view that the chief function of visual stimuli in the form of sex display and courtship phenomena among various classes of animals is to promote an effective synchronization of the male and female sexual processes He suggested that this synchronization is mainly effected by pituitary stimulation through the intermediation of the hypothalamus

It is well known that for any one species of bird the number of eggs in a clutch is generally constant within narrow limits The bird tends to lay a definite number of eggs and then brood over them If eggs are removed one or two at a time as they are laid the bird may continue to lay almost indefinitely For example Davie in 1889 induced a flicker to lay 71 eggs in 73 days in this way, and Witschi (1935) by removing eggs daily from the house sparrow induced the bird to lay up to 50 eggs in succession (see Marshall 1936) Since the interval between ovulation and the laying of the egg appears to be about a day the repetition of ovulation in these cases seems to be due to some exteroceptive stimulus though whether the stimulus acts through the eye or through tactile receptors on the ventral body surface is unknown

In most birds and some mammals the occurrence of ovulation in the breeding season is dependent on some stimulus received from the male This phenomenon has been studied in the pigeon by Matthews (1939) He found that the isolated female pigeon did not ovulate though if the bird was confined with or in view of a male another female or a mirror then ovulation and oviposition would occur This latter observation is of interest in that the reflected image from the mirror must have acted as a stimulus through the optic pathway but in this case the stimulus would seem to be a visual pattern rather than quantity of light Among the mammals the rabbit ferret cat ground squirrel short tailed shrew and mink require some stimulus normally supplied by coitus, for ovulation to occur This fact was first reported for the rabbit by Heape (1905) Much early work excluded such factors as absorption of semen by the female tract or a nervous

reflex acting on the ovaries from playing a part The evidence strongly suggests that post coital ovulation is dependent on reflex excitation of the anterior pituitary by the act of coitus with resultant discharge of gonadotrophic hormone A similar mechanism is probably involved in the development of pseudo pregnancy in the rat following sterile coitus In this form ovulation occurs spontaneously, but the corpora lutea so formed do not become functionally active unless coitus has occurred Occasionally rats may show failure of ovulation and enter a state of persistent oestrus Everett (1939 1940) has studied a strain of rats in which this abnormality was common and found that when a female rat in persistent oestrus accepts coitus with the male ovulation and the recurrence of cycles may follow

The nervous pathway involved in reflex excitation of the pituitary is not clear Since mechanical or electrical stimulation of the vulva vagina or uterine cervix may result in ovulation in the rabbit or a pseudopregnancy response in the rat it seems likely that the sensory receptors lie mainly in this area but the fact that local anaesthesia of the vulva and vagina does not prevent ovulation after coitus (Fee and Parkes 1930) indicates that other receptors play an important part The work of Brooks (1937) who showed that removal of the olfactory lobes and neo cortex or destruction of the labyrinths and cochleae in the female rabbit does not prevent mating and subsequent ovulation also indicates that the stage of sexual excitement necessary to cause secretion of pituitary gonadotrophins may be produced by stimuli from a variety of sensory end organs It is probable that afferent nerve impulses from the reproductive tract however exert an important influence on the time of ovulation in many forms For example in two rhythmically ovulating forms the sheep and the fowl mechanical irritation of the reproductive tract may markedly affect the cycle of events Moore and Nalbandov (1953) found that mechanical distension of the uterine cornu of the ewe shortened the oestrous cycle from an average of 16 3 days to 11 9 days The maintenance of normal cycles (16 1 days) in ewes following distension of a denervated segment of the uterus indicated that this phenomenon is mediated over nervous pathways In the fowl about 25 hours elapse between ovulation and the laying of the finished egg and in hens laying daily ovulation occurs 30–60 minutes after oviposition Ovula

tion does not usually occur while there is an egg in any part of the oviduct Huston and Nalbandov (1953) observed that the presence of an irritant such as a loop of thread in the magnum part of the oviduct completely suppressed ovulation in the great majority of birds From their evidence these workers suggest that the presence of the thread neurogenically inhibits the peak levels of L H secretion necessary for ovulation and think that this mechanism is of physiological importance in serving the hen as a timing device which prevents ovulation while a yolk (an irritant) is passing through the oviduct

There is much evidence which will be discussed below that reflex paths from many sensory receptors converge in the hypo thalamic region and excite the anterior pituitary gland by a path through the hypophysial stalk

Clinical data

There are so many reports describing changes in the menstrual cycle associated with emotional upsets that there can be little doubt of their overall validity Theobald in 1936 summarized the position at that time and discussed the fact that the men strual cycle may be influenced by hypnosis as well as by psycho logical factors due to change of climate, change of occupation shocks and excitements of all kinds Many other accounts could be quoted such as that of Fuerstner (1944) who described the amenorrhoea produced by fear of pregnancy in unmarried girls and Bear (1943) who suggested excessive desire for a child may contribute to sterility In this latter connection Bear mentions the possibility that adoption of a child removes the psychological factor involved which could explain the frequently described occurrence of pregnancy following adoption Loeser (1943) ob served several cases of amenorrhoea following bombardment during the last war Following the missed period in these women pieces of endometrium were removed and examination of this tissue was said to show that the uterine cycle had ceased at the time of the fright

The most likely hypothesis to account for the fact that extero ceptive and psychological factors determine and modify the breeding oestrous and menstrual cycles of so many forms is that the central nervous system controls the secretion of gonado trophic hormone from the pituitary gland An alternative view

that the nervous system regulates the gonads by a secreto motor nerve supply finds little support The gonads certainly receive *nerve fibres but it is likely that these are vasomotor and sensory* in nature only (Kuntz 1919 and 1929 Kuntz and Morris 1946 Gray 1947 Koppen 1951) The view of most workers is that the greater number of nerve fibres to the gonads accompany and innervate the blood vessels Some nerve filaments end in the tunica albuginea and vasculosa of the testis or fibromuscular tissue of the ovary but it is doubtful if any fibres end on the seminiferous or interstitial cells of the testis or the follicular or interstitial cells of the ovary Experimental data support these conclusions regarding the lack of a secreto motor innervation to the gonads for as mentioned in Chapter 3 transplanted ovaries maintain normal oestrous cycles Further exteroceptive stimuli *such as coitus in the rabbit can still produce ovarian responses in the transplanted organ (Friedman 1929) There can be little* doubt then that exteroceptive factors affect the ovary through the intermediation of the pituitary gland

The Hypothalamus and Gonadotrophic Secretion

Since the anterior pituitary gland of the embryo (Rathke s pouch) migrates from the roof of the mouth to become at ached to the floor of the third ventricle it would seem likely on teleo logical grounds that the hypothalamus is the part of the central nervous system most directly concerned with the regulation of anterior pituitary activity Such a relationship has been investi gated by experiments in which the hypothalamus has been electrically or pharmacologically stimulated and in which lesions *have been placed in various parts of the hypothalamus or in the* pituitary stalk

Electrical stimulation of the hypothalamus

Stimulation of the hypothalamus with observation of any resultant increase in gonadotrophic secretion is a difficult pro cedure owing to the nature of reproductive phenomena The slow rate of onset of puberty and reproductive development at the beginning of the breeding season the relatively slow rhythm of the oestrous cycle and the slow maturation of follicles and corpora lutea makes it seem unlikely that short lived stimulation of the hypothalamus would produce an observable ovarian reac

tion One of the fastest ovarian responses known is that of ovulation in the rabbit (and similar forms) which normally occurs 9–11 hours after the act of coitus Fee and Parkes (1929) showed that if the pituitary gland is removed from a rabbit within one hour of coitus the subsequent ovulation is prevented but hypophysectomy later than this is followed by ovulation It appears that the nervous stimulus of mating reflexly excites the discharge of gonadotrophin, and that sufficient gonadotrophin has been released into the blood stream within one hour to cause rupture of the graafian follicles some 9 hours later This quick response on the part of the rabbit s pituitary and ovary has been much used in studies concerning gonadotrophic secretion

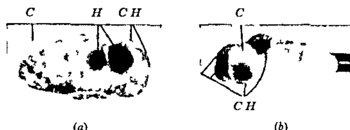


Fig 32 Cystic follicles in the ovaries of rabbits consequent upon electrical stimulation of the hypothalamus
C cystic follicles *CH* cystic follicles becoming haemorrhagic *H* haemorrhagic follicles × $\frac{3}{2}$
(From Harris G W (1937) *Proc Roy Soc B* 122 379)

The basic experiment dealing with nervous stimulation and gonadotrophic release in the rabbit was made by Marshall and Verney (1936) when they showed that diffuse electrical stimuli applied to the head or the lumbar spinal cord of rabbits resulted in ovulation and pseudopregnancy in a large proportion of animals Similar results were then obtained in rats (Harris 1936) in which functionally active corpora lutea and a state of pseudo pregnancy were induced by diffuse electrical stimuli applied to the head These results seemed to indicate that excitation of some neural structure had occurred and that this in turn had stimulated the anterior pituitary In an attempt to delimit this neural structure more closely localized electrical stimuli were applied to different regions of the hypothalamus and pituitary gland in anaesthetized rabbits (Harris, 1937) In this work it was

found that stimulation of the tuber cinereum posterior hypothalamus, or pituitary gland directly might result in ovulation or the formation of cystic or haemorrhagic follicles (a result which often follows the injection of anterior pituitary like principles—Fig 32) These results were soon confirmed in the main by the work of Haterius and Derbyshire (1937) More recently two independent researches have shown that electrical stimuli which are ineffective in causing gonadotrophic discharge if applied directly to the pituitary gland are fully effective in producing this result if applied to the tuber cinereum Markee Sawyer and Hollinshead (1946) working on rabbits anaesthetized with ether stimulated the pituitary by a pharyngeal or temporal approach and the hypothalamus through the superior orbital fissure They found that electrical stimulation of the pituitary did not result in ovulation unless there were signs of spread of the stimulus whereas stimulation of the hypothalamus at a lower voltage resulted in ovulation in three out of four animals Harris (1948) found that remote control stimulation of the tuber cinereum in the unanaesthetized rabbit for as short a period as 3 minutes, may be followed by a full ovulatory response whereas similar stimuli applied to the anterior pituitary pars intermedia or infundibular stem for periods up to 7½ hours were not followed by any ovarian response It is likely that the earlier results quoted above in which ovulation followed stimulation of the pituitary gland directly were due to the complications induced by anaesthesia

The remote control method of stimulation possesses many advantages and some disadvantages It allows the application of electrical stimulation without the use of anaesthesia and therefore stimulation may be performed for prolonged periods It permits stimulation without concomitant operative trauma It also makes possible the repetition of an experiment several or many times in the same animal and thereby reduces the chances that variable factors such as differences in the nutritional or oestrous state of the animal have influenced the result The main disadvantage is the difficulty of controlling the shape of the stimulating pulse Since this technique has been found of much value in studying the effects of hypothalamic stimulation on pituitary function (see also Chapters 5 9 10 and 11) the method will be briefly described

In a preliminary operation a small flat coil of 2 000 turns of enamelled copper wire is buried beneath the scalp but outside the skull of a rabbit The inner turn of the coil is soldered to a glass insulated platinum electrode carried on an ebonite bush which is shaped and flanged to plug firmly into a hollow screw in the skull The outer turn of the coil is soldered to either a



Fig 33 X ray photograph of the head of a rabbit with the coil unit *in situ* The coil unit was inserted on 2nd January 1941 and the X ray photograph taken on 14th January 1944 × ¹

(From Harris G W (1947) *Philos Trans B* 232 385)

german silver plate or to four turns of uninsulated stainless steel wire which act as the indifferent electrode Fig 33 shows an X ray photograph of a rabbit s head with such a coil unit in position It may be noticed that the coil in lateral view appears flat in shape that the stimulating electrode passes downwards through the midline structures of the brain so that the bare tip is situated in the hypothalamus and that the indifferent elec trode is placed over the frontal bones The only part of the unit

in contact with intracranial tissue is the insulating glass capillary tube and the bared 1 mm of platinum wire Neither of these substances causes any microscopically visible reaction in the tissues of the brain or pituitary gland After recovery from the initial operation stimulation may be performed by placing the animal s head in an electromagnetic field and inducing a voltage



(*a*) (*b*)

Fig 34 Microphotographs of horizontal sections through the pituitary gland and skull base of a rabbit to show the track left by an implanted electrode In the region of the insulated part of the electrode (*b*) the electrode track is of greater diameter than in the region of the bared tip (*a*) × 10
(Harris G W unpublished)

in the buried coil For short periods of stimulation this may conveniently be performed by holding the animal s head in relationship to a primary (external) coil carrying a pulsating current (Harris 1947) For longer periods of stimulation the animal may be restrained in a hammock and a primary coil suspended at a measured distance over the animal s head (Harris 1948) or alternatively the animal inside its usual cage may be placed inside a large primary coil some 3 feet in diamater and

large current pulses passed through this coil (de Groot and Harris 1950) Using this technique rabbits have been stimulated for 3 hours daily for one week and for as long as 48 hours continuously and have been kept alive without any detrimental effects for periods up to 3 years When the experiment is completed the animal is killed and the vascular system perfused with formalin with the electrode still *in situ* After sectioning the brain the electrode track is clearly visible as a circular hole in the brain tissue the diameter of the hole being smaller in the region of the uninsulated tip of the electrode (see Fig 34) This allows the exact site of the stimulated tissue to be ascertained

The remote control method has been used by many workers for stimulating different regions of the central nervous system Two different methods have been used however, for inducing the stimulating pulses from the external primary coil to the implanted secondary coil (Fig 35) Fender (1941) Greig and Ritchie (1944) Lafferty and Farrell (1949) and Hume (1953) pass a high frequency current (about 100–430 kc /sec) modulated at a lower frequency (about 60 c /sec) through the primary coil The buried unit which in these cases consisted of a coil small metal rectifier and condensers picks up the high frequency pulses but these are rectified so that the final stimulus to the tissues is effectively that of square wave pulses at the modulating frequency The method has the advantage that the necessary voltage is easily induced into the implanted unit but has the disadvantage that there is a greater risk of polarization and gas formation at the electrode tip with consequent damage to nervous tissue The second method of inducing the stimulus (Loucks 1934 Chaffee and Light 1934 Clark and Ward 1937 and Harris 1947) is to pass relatively low frequency pulses through the primary coil and to use the same frequency pulses in the secondary coil for stimulation With this technique the peak current necessary in the primary coil is higher but there is less risk of chemical changes and damage occuring at the stimulation site since a c instead of d c pulses are applied to the tissues It is felt that this second method is safer especially when the electrode is situated in solid brain tissue rather than on a surface of the brain One further factor of importance is the limited spread of current when this method of induction is used in the unanaesthetized animal On applying such stimulation

with the electrode tip in the vicinity of the pituitary stalk it is possible to see elevation of the upper eyelid and rotatory movements of the eyeball due to spread of the stimulus to the oculo motor nerve With stronger stimuli the vibrissae move the jaw muscles contract and movements of the ears neck muscles and forelimb muscles occur due to spread of stimulus to the

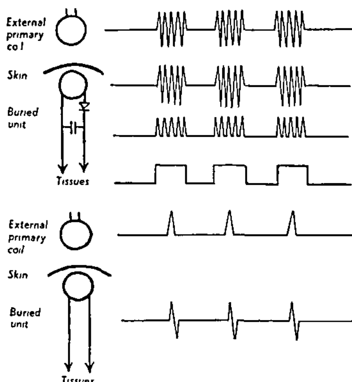


Fig 35 To illustrate the wave forms or effective wave form in the primary coils or implanted units in the two methods of applying remote control stimulation

medial fibres of the internal capsule Stimuli strong enough to elicit these latter reactions are only applied under anaesthesia Unless the electrode is placed in close relationship to the optic chiasma or tract or more dorsally in the hypothalamus near the anterior thalamic nuclei stimuli strong enough to cause marked movements of the orbital structures may be applied without any

large current pulses passed through this coil (de Groot and Harris 1950) Using this technique, rabbits have been stimulated for 3 hours daily for one week, and for as long as 48 hours continuously and have been kept alive without any detrimental effects for periods up to 3 years When the experiment is completed the animal is killed and the vascular system perfused with formalin with the electrode still *in situ* After sectioning the brain the electrode track is clearly visible as a circular hole in the brain tissue, the diameter of the hole being smaller in the region of the uninsulated tip of the electrode (see Fig 34) This allows the exact site of the stimulated tissue to be ascertained

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case insufficient to cause observable discharge of follicle stimulating hormone As far as is known no studies have been made of the effect of hypothalamic stimulation on the release of this hormone probably because the response would be of a much slower nature Such an investigation would be possible however using the remote control method on an animal which has a definite period of seasonal anoestrus

Pharmacological activation of the hypothalamus

Several workers have investigated the effects of different pharmacological agents injected intravenously in rabbits in producing gonadotrophic secretion and ovulation Fevold Hisaw and Greep (1936) found that intravenous injection of copper acetate in oestrous rabbits resulted in ovulation Marshall Verney and Vogt (1939) noted that intravenous injection of picrotoxin in near convulsant producing or convulsant producing doses resulted in ovulation or the production of cystic and haemorrhagic follicles in the ovaries of rabbits Similar injections of strychnine HCl apomorphine HCl β tetrahydronaphthylamine ergometrine carbaminoylcholine HCl and coriamyrtin had no effect Pilocarpine eserine acetyl choline and adrenaline had been found ineffective in this respect by Marshall and Verney (1936) in earlier work Intravenous injection of cadmium salts but not of salts of barium cobalt gold iron manganese nickel silver or zinc (Emmens 1940) or intravenous injection of metrazol but not of strychnine insulin benzedrine or ephedrine (Brooks Beadenkopf and Bojar 1940) or intravenous injection of various plant juices (Bradbury 1944) have also been found to result in ovulation Some of these drugs have been found to produce pseudopregnancy if injected into oestrous rats (Dury and Bradbury 1942) but since it has recently been shown that a wide variety of injected substances will also result in the same reaction (Swingle Seay Perlmutt Collins Barlow Junr and Fedor 1951) this response does not seem to be of a very specific nature In investigating these reactions in the rabbit most workers have used copper salts picrotoxin or metrazol At first it was thought possible that these substances produced follicular rupture by potentiating or augmenting the action of the endogenous gonadotrophin already in the circulation This now seems unlikely for it has been found that transection of the hypo

sign of an emotional response on the part of the animal At this strength of stimulation the current spreads several millimetres to affect the myelinated fibres of the oculomotor nerve which have a low threshold of excitation but the effective spread for the un myelinated fibres of the hypothalamus is not more than ½ mm This latter fact has been clearly established by study of the secretion of the antidiuretic hormone in animals in which the electrode tip is placed at varying distances from the supraoptico hypophysial tract It is also noteworthy that although move ments of the eyeball may be produced easily by such stimulation in this region changes in diameter of the pupil are not observed indicating that the autonomic fibres of the oculomotor nerve are not excited at a current strength which affects the somatic fibres

Returning to the effect of electrical stimulation in causing gonadotrophic secretion and ovulation the position seems to be that stimulation of the tuber cinereum may produce this response whilst similar stimulation applied to the pituitary stalk or to the pituitary gland directly is ineffective For this reason, and on the basis of the anatomical arrangements of the structures in volved it is likely that the stimulus from the hypothalamus is transmitted through the pituitary stalk but by structures that are not excitable to electrical stimulation The component of the stalk which appears to fulfil these requirements is the hypo physial portal vessels and from the above results it seems likely that stimulation of the hypothalamus excites nerve fibres which transmit the stimulus to the pituitary gland via these vessels of the stalk If the last link in the chain of events leading to gonado trophic release is humorally mediated by the portal vessels it is easy to understand why electrical stimulation of the pituitary stalk and gland does not evoke such secretion In this respect it is of interest that the denervated adrenal medulla is almost in excitable to direct electrical stimulation (Cannon and Rosen blueth 1937)

Little work has been performed on other forms on the effect of localized stimulation of the hypothalamus in causing secretion of gonadotrophic hormone The basis of the ovulation response in the rabbit seems to be the secretion of luteinizing hormone Stimulation of the tuber cinereum for short periods (1 hour) in rabbits which were anoestrous did not provoke any ovarian response but it is likely that the period of stimulation was in any

and other lesions in the hypothalamus result in lack of development of the sex glands (see Brooks 1940) Much more significant however are the experimental findings that hypothalamic damage may result in disturbances of the cycle such as persistent oestrus in a normally cyclic species and in the clinical finding that hypothalamic tumours may produce a state of precocious puberty This latter condition is a more highly specific response than that of hypogonadism In 1941 Weinberger and Grant described such a case and reviewed seventeen others in the literature The conclusions they reached were that pineal tumours may result in precocious puberty if they involve secondarily the posterior hypothalamic region and that small highly localized tumours of the hypothalamus in the region of the tuber cinereum or mammillary bodies may result in pubertas praecox by damaging structures which normally inhibit the release of pituitary gonadotrophin It is of interest that some of the young children they described showed behavioural changes as well as an endocrine condition typical of the adult Bauer (1954) has recently brought the literature on this subject up to date in a survey of twenty four cases of precocious puberty due to a hypothalamic lesion In all cases the presence of a normal pituitary gland was described

Hypothalamic lesions may affect reproductive phenomena in two ways Firstly the lesion may damage some nervous structure necessary for integrating the patterns of behaviour involved in oestrus mating or maternal activities In other words an animal may show the endocrine changes typical of oestrus but refuse to mate since the reflex arcs involved in mating behaviour are broken or incoordinated Studies of this type are usually made on gonadectomized animals under controlled hormonal administration and are discussed further in Chapter 12 The second way in which hypothalamic lesions may affect reproductive phenomena is by damage to the nervous structures which regulate the release of gonadotrophins In the intact animal these two activities of the hypothalamus are probably closely interrelated

Dey and his co workers in a series of papers (see Dey 1943) have described the effects of hypothalamic lesions on the reproductive organs of guinea pigs In a large group of female guinea pigs they found that animals with lesions destroying the major

physial stalk before or shortly after the injection of copper acetate picrotoxin or metrazol inhibits the usual ovulatory response (Brooks Beadenkopf and Bojar 1940) and also that copper acetate injected into the third ventricle of the brain in one two-hundredth the amount required intravenously is effective in a high proportion of rabbits (Harris 1941) It therefore seems that these substances act by stimulating some nervous mechanism probably in the hypothalamus which in turn stimulates the anterior pituitary gland Recently however, Sawyer and Markee (1950) have produced evidence which they believe indicates that picrotoxin acts on some neural structure, but that copper acetate acts directly on pituitary cells These workers found that rabbits previously treated with dibenamine or atropine failed to ovulate if treated with picrotoxin and that nembutal in anaesthetic doses blocked the action of 2 to 4 times the minimal ovulating dose of picrotoxin On the other hand, intravenously administered copper acetate produced ovulation after previous administration of dibenamine atropine or nembutal Further copper acetate injected directly into the pituitary gland in doses of 0 15 mg in 0 15 ml (4 injections at 5–10 min intervals of 0 04 ml each) produced ovulation in 6 out of 8 animals Sawyer and Markee suggest that the results of Harris (1941) were due to the copper acetate (which was effective in doses of 0 05 mg in 0 05 ml when injected into the third ventricle) being carried by the hypophysial portal vessels to act on the anterior pituitary gland The results of Brooks et al (1940) however are difficult to understand if the copper salt acts directly on anterior pituitary cells It is possible though that a toxic substance, such as copper acetate may activate both nerve cells and gland cells if in sufficient concentration

Hypothalamic lesions

It has long been known that hypothalamic lesions may result in genital atrophy (Camus and Roussy 1920 Bailey and Bremer, 1921 Smith 1927) The conclusion to be drawn from such observations however is uncertain The hypothalamus is anatomically so small in size and consists of such an intricate mass of nerve fibres and scattered cell groups that even a small discrete lesion may affect many functions (metabolic vascular etc) which could indirectly cause gonadal atrophy The same considerations apply to the many clinical observations that tumours

reproductive activity following the operation of pituitary stalk section and highly discordant results have been obtained The essential control procedure of checking the stalk section post mortem by serially sectioning a block of tissue containing the pituitary gland and hypothalamus is not mentioned in some of the published reports Examination of the region under a dissecting microscope is insufficient control of the completeness of the operation However allowing for inadequate technique there are still discrepancies in the results obtained and a few of these will be mentioned to illustrate the position In rats, pituitary stalk section was found by Richter (1933) and Brooks (1940) to produce lengthened oestrous cycles Westman and Jacobsohn (1937, 1938) and Brolin (1946) reported gonadal atrophy in male and female rats whereas Dempsey and Uotila (1940) and Dempsey and Searles (1943) observed normal lengthened or absent oestrous cycles in stalk sectioned animals Transection of the stalk in guinea pigs was found by Dempsey (1939) and Leininger and Ranson (1943) to be followed by normal irregular or absent oestrous cycles In rabbits stalk section has been followed by gonadal atrophy (Harris 1937, Hinsey 1937 Westman and Jacobsohn 1940) and by the recurrence of oestrus (Brooks 1938) In dogs silver clips applied to the pituitary stalk (Mahoney and Sheehan 1936) or division of the stalk (Dott 1923) caused gonadal atrophy while other workers (Keller and Hamilton 1937) reported normal sex functions in the bitch following stalk section Breckenridge and Keller (1948) found surgical extirpation of the hypophysial stalk in 9 dogs was followed by atrophic ovaries and genital tracts in 3 dogs and normal organs in six In the human Dandy (1940) has described a case of stalk section in a young woman that was followed by normal menstrual cycles pregnancy labour and lactation

In drawing conclusions from these results most workers have assumed that any hypothalamic influence transmitted down the stalk to the adenohypophysis would traverse nerve fibres Since the nerve fibres of the stalk lack a neurilemmal sheath and strictly speaking are nerve fibres of the brain rather than of a peripheral nerve they do not regenerate once they are severed On this assumption many workers have tended to one of two points of view in explaining the variable results of stalk section Firstly it has been suggested that section of the stalk does not

part of the median eminence became acyclic and developed genital atrophy whilst animals with lesions at the caudal end of the optic chiasma showed only large follicles in the ovaries hypertrophied genital organs and permanently open vaginae, which they interpreted as due to lack of secretion of the luteinizing hormone Over forty animals with lesions elsewhere in the hypothalamus showed no disturbance of oestrous cycles, though many would not mate Similar results have been obtained in rats Hetherington and Ranson (1940) described obesity and sexual dystrophy following lesions in the tuber cinereum of these animals More recently Hillarp (1949) has reported further results obtained on rats and largely confirmed the results of Dey (1943) He found that bilateral lesions placed anterior and ventral to the paraventricular nucleus resulted in a state of constant oestrus The same result was obtained by means of much smaller lesions placed caudal to the paraventricular nucleus and it was suggested that this was due to the interruption of a fairly well demarcated fibre system arising in the anterior hypothalamic area and running superficially on both sides of the median eminence towards the hypophysial stalk

From these and other studies it would appear that the hypothalamus is intimately concerned with the regulation of gonadotrophic secretion by the adenohypophysis but the exact localization of the nerve cells and fibres concerned is unknown There is also little information available regarding the differential control of the follicle stimulating luteinizing and luteotrophic hormones

Pituitary Stalk and Gonadotrophic Secretion

The anatomical pathway by which the hypothalamus regulates anterior pituitary secretion has been the subject of much conjecture Since the hypothalamus is known to influence the sympathetic and possibly the parasympathetic nervous systems many workers investigated the effect of removing peripheral autonomic pathways on gonadotrophic secretion It is now clear that the cervical sympathetic system and the petrosal nerves exert little effect on anterior pituitary activity (see Chapter 2) and attention has become focused on the participation of the pituitary stalk

Many investigators have studied gonadotrophic secretion and

examination of the hypothalamus and pituitary gland showed regeneration of the portal vessels between the median eminence and adenohypophysis through the interstices of the wool pledgets In 19 other animals small plates of paper were inserted between the cut ends of the stalk Eleven of these remained anoestrus gonadal atrophy occurred and histological examination showed absence of portal vessel regeneration in 8 irregular or regular oestrous cycles recurred and after death some regeneration of the portal vessels was observed around the edge of the (misplaced) plates In further animals killed 1–10 days after stalk section regeneration of the portal vessels was seen to commence within 1–2 days It is unlikely that reduction in the amount of anterior lobe tissue consequent on a diminished total blood supply was responsible for the post operative disturbances in oestrous cycles in this work for the following reasons

(i) Smith (1932) showed that as little as 10 per cent of the normal amount of anterior pituitary tissue in rats is sufficient to maintain some ovarian activity and that 30 per cent can maintain normal sexual functions
(ii) The post operative abnormality or otherwise of the reproductive processes bore no relation to the amount of anterior lobe tissue present (see also Breckenridge and Keller 1948)
(iii) The indian ink injections showed that the pars distalis was still well vascularized in the animals in which portal vessel regeneration was completely prevented and which remained anoestrus after operation

The conclusion drawn from this work was that the post operative return of ovarian activity could be correlated with regeneration of the portal vessels

The results of Greep and Barrnett (1951) and Barrnett and Greep (1951) who studied the effects of pituitary stalk section in male and female rats are believed to support the above conclusions (though this is not the view of the authors) These workers sectioned the pituitary stalk by the parapharyngeal route and found that in 43 female rats 14 showed absence of cycles 21 rats showed one period of vaginal oestrus and only 8 animals had oestrous smears on two to five separate occasions in the post operative period Histological study of the ovaries of these

necessarily affect the pituitary secretion of gonadotrophin and that any gonadal atrophy is due to incidental interference with the pituitary blood supply A second point of view is that animals which show normal reproductive functions after operation may be best explained on the grounds of incomplete stalk severance and that complete section is inevitably followed by gonadal atrophy It is this latter view which has been taken by most investigators who have serially sectioned the pituitary stalk region of their animals and discarded those cases in which any tissue connected the two cut ends of the stalk A possible reconciliation of these variable results became apparent when it was found that the hypophysial portal system of vessels formed a constant anatomical feature of the pituitary stalk and that the flow of blood in these vessels was from the median eminence of the hypothalamus towards the adenohypophysis It then seemed possible that the influence of the hypothalamus on gonadotrophic secretion was mediated via these vessels and that the variable results of pituitary stalk section might be explained in terms of variable degrees of portal vessel regeneration across the site of section To test this hypothesis a temporal approach to the rat s pituitary gland was developed and the operation of stalk section performed in a series of 23 female animals (Harris 1950) Post operatively one animal remained anoestrous till killed and was found to have atrophic reproductive organs 8 animals showed irregular oestrous cycles and 14 rats regular cycles After death the vascular system was perfused with indian ink and serial sections cut through a block of tissue containing the hypothalamus pituitary stalk and gland It was found that the hypophysial portal vessels had regenerated to some extent in all cases and that the amount of regeneration could be roughly correlated with the degree of reproductive activity (oestrous cycles and a pseudopregnancy response to sterile coitus) observed after operation

In a further series of rats the stalk was divided and foreign bodies inserted between the cut ends to see the effect of preventing regeneration of the portal vessels In the first ten experiments pledgets of cotton or celloidon wool were used but it was observed that these pledgets swelled considerably after insertion thus producing damage to the hypothalamus However it was of interest that 6 of these animals showed a return of irregular or regular oestrous cycles after operation and that microscopic

examination of the hypothalamus and pituitary gland showed regeneration of the portal vessels between the median eminence and adenohypophysis through the interstices of the wool pledgets In 19 other animals small plates of paper were inserted between the cut ends of the stalk Eleven of these remained anoestrus gonadal atrophy occurred and histological examination showed absence of portal vessel regeneration in 8 irregular or regular oestrous cycles recurred and after death some regeneration of the portal vessels was observed around the edge of the (misplaced) plates In further animals killed 1–10 days after stalk section regeneration of the portal vessels was seen to commence within 1–2 days It is unlikely that reduction in the amount of anterior lobe tissue consequent on a diminished total blood supply was responsible for the post operative disturbances in oestrous cycles in this work for the following reasons

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animals revealed gonadal atrophy In their studies on male rats they found atrophy of the testes seminal vesicles adrenals and thyroid glands and decreased growth following stalk section After death the vascular system of some of these male and female rats was perfused with indian ink and serial sections cut through a block of tissue containing the hypothalamus and pituitary gland A study of these sections failed to detect regeneration of the portal vessels and the comment is made observations of the operative site in our instances invariably showed that the stalk was buried in scar tissue and adherent to the anterior lip of the bony incision a fact which could account for lack of regeneration of vascular channels This fact might also account for failure to observe minor degrees of regeneration However there would seem to be a fundamental difference so far as the future history of the pituitary stalk is concerned according to whether it is sectioned by the temporal route or the parapharyngeal route In the first case the two cut ends of the stalk are left lying in their normal environment surrounded by cerebrospinal fluid and smooth meninges, whereas in the second case they are left in contact with the rough surface of the trephine hole in the base of the skull and in this position they become involved in the scar tissue that develops in the region Greep and Barrnett draw the conclusion from their experiments that the gonadal atrophy that followed pituitary stalk section in their animals was due to interference with the total blood supply to the pituitary gland and not necessarily to interference with a hypophysial portal blood supply It is difficult to reconcile this view with Greep s previous findings (Greep 1936) In this earlier work Greep hypophysectomized 28 day old male and female rats and then replaced either the animal s own gland or the glands from other animals in the sella turcica Following this procedure a high proportion of the animals showed evidence of normal anterior pituitary activity as demonstrated by growth rate oestrous cycles pregnancy and lactation It is clear that in these cases the anterior pituitary tissue must have suffered a greater interference with its total blood supply than in the experiments involving stalk section On the other hand there would seem to be more favourable conditions for the regeneration of a portal blood supply in the grafted animals than in the animals with their stalks sectioned by the *parapharyngeal* route

Several recent reports have dealt with the effect of extra illumination in winter in producing oestrus in pituitary stalk sectioned ferrets As mentioned above the effect of extra light in producing oestrus in the normal ferret is mediated by the eyes optic nerves and probably some nervous reflex pathway ultimately leading to increased pituitary secretion of gonadotrophic hormone In order to see whether the pituitary stalk formed part of the anatomical pathway involved Thomson and Zuckerman (1953) severed the pituitary stalk in 17 ferrets by a buccal approach and exposed them to a light stimulus in winter They believe that 2 of these animals which reacted to light exposure by becoming oestrus did so in the absence of any vascular connections between the median eminence and the adenohypophysis They conclude that the hypophysial portal vessels do not form part of the pathway by which light stimulates anterior pituitary secretion in the ferret This work has been repeated by Donovan and Harris (1954) who obtained different results They exposed the pituitary stalk of 24 ferrets by a temporal approach In 4 animals the stalk was left intact in 6 simple stalk section was performed and in 14 the stalk was cut and a paper plate inserted between the hypothalamus and pituitary gland Twelve of these 24 ferrets became oestrus when exposed to prolonged illumination These included the 4 blank operated control animals 5 of the 6 subjected to simple stalk section and 3 in which a paper plate had been inserted After killing the vascular system was perfused with indian ink and celloidin sections cut serially through the pituitary region at a thickness of 130–200 μ (usually 160 μ) Histological examination showed that (*a*) the pituitary stalk had been successfully sectioned in all but the blank operated animals (*b*) the appearance of oestrus could be correlated with the presence of vascular connections between the median eminence of the tuber cinereum and anterior pituitary gland no case of oestrus was observed in the absence of such vascular connections (*c*) the anoestrous animals had few if any vascular connections between the median eminence and anterior pituitary gland and (*d*) some atrophy of the pituitary gland especially of the posterior lobe occurred in all animals in which the stalk was sectioned However as shown in Table I the extent of the atrophy was not related to the response of the animals to light (cf Greep and Barrnett 1951 Barrnett and Greep 1951)

TABLE I

To show the relative volume of the whole pituitary gland and of the anterior and posterior lobes in ferrets in which the pituitary stalk had been cut

	Number in Group	Total Volume	Anterior Lobe	Posterior Lobe
Blank operated (oestrus)	4	100	100	100
Stalk cut (anoestrus)	10	73	95	45
Stalk cut (oestrus)	8	61	80	39

From these results it was concluded that the hypophysial portal vessels form a necessary part of the pathway of the light induced *oestrous response in the ferret* *In comparing the results and* conclusions of Thomson and Zuckerman (1953) and Donovan and Harris (1954) the following points may be borne in mind

(i) Thomson and Zuckerman base their conclusions on results obtained in only two ferrets

(ii) The surgical approach used by Thomson and Zuckerman (buccal route) gives poor facility for placing plates between the cut ends of the stalk and the plates were with a few exceptions eliminated from the wound As pointed *out above this operation often results in anatomical dis* organization of the pituitary stalk region which complicates the final histological study

(iii) Thomson and Zuckerman made their microscopic study of serial sections through the pituitary hypothalamic region after imbedding the specimens in wax and sectioning at 10 μ To obtain a clear picture of the vascular anatomy it is necessary to cut thick serial sections (160 μ) after celloidin imbedding

(iv) Donovan and Harris (1954) report that through the kindness of Dr Thomson and Professor Zuckerman they had

the opportunity of examining sections through the pituitary region of one of their two critical ferrets Inspection revealed that a significant proportion of the visible blood vessels did not contain indian ink Conclusions drawn from incompletely injected specimens must be regarded as unreliable

These technical points have been mentioned here in some detail since they are of basic importance to investigations in this field

One other work dealing with the relationship between pituitary stalk lesions and gonadotrophic secretion should be mentioned Benoit and Assenmacher (1954) have reported the results of their experiments on the duck In this form the anterior and posterior lobes of the pituitary gland are separated by a septum so that discrete lesions may be made in either the portal vessels of the stalk *or* the nerve fibres of the stalk Benoit and Assenmacher found that cutting the portal vessels and placing a plate of sclera between the median eminence and pars distalis resulted in atrophy of the testes and failure of the testicular response to light A similar condition followed injury to the anterior part of the median eminence whereas section of the nerve fibres of the pituitary stalk (leaving the portal vessels intact) was compatible with normally developed gonads

The conclusion drawn from the experimental data discussed above is that section of the pituitary stalk affects the functions of the anterior pituitary by interrupting a vascular supply the portal vessels which exerts some specific effect on activity of the gland This conclusion is strongly reinforced by that drawn from the experiments dealing with grafts and transplants of anterior pituitary tissue described in Chapter 3 These experiments also indicate that good vascularization irrespective of the source of the blood is not sufficient to render anterior lobe tissue functional some additional factor is necessary and this factor is present when the blood supply is derived at least in part from the hypophysial portal system

Neural Origin of Oestrous Cycles Puberty and Sexual Differentiation of the Hypothalamus

Oestrous cycles

The view first expressed by Moore and Price (1932) regarding

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(iv) Donovan and Harris (1954) report that through the kindness of Dr Thomson and Professor Zuckerman they had

which is inhibited by a blood level of oestrogen above a certain threshold value These two views may be represented as in Fig 36 It is now clear that the central nervous system participates in some way in these reactions for the following reasons

(*a*) Lesions in the anterior hypothalamus of the guinea pig and rat may disturb the rhythmic release of the luteinizing hormone and result in a state of constant oestrus

(*b*) Section of the pituitary stalk with precautions to prevent regeneration of the hypophysial portal vessels brings all reproductive cycles to a halt and results in gonadal atrophy

(*c*) Anterior pituitary transplants in a site remote from the sella turcica in hypophysectomized animals do not maintain oestrous cycles Similar grafts placed under the median eminence of the hypothalamus where they become revascularized by the portal vessels maintain normal reproductive functions Even anterior pituitary tissue from a male animal will maintain *cyclical* activity if transplanted to this latter site in a female host

Since massive doses of oestrogens are claimed by many investigators to induce cytological changes in anterior pituitary cells of transplanted or stalk cut glands it is possible that oestrogens exert some direct action on the adenohypophysis but there can be little doubt that the main action of oestrogens in the regulation of cycles is exerted on the central nervous system The beautifully precise experiments performed by Everett Markee and Sawyer on their 4 and 5 day cyclic strains of rats lends strong support to this view By working with inbred Vanderbilt strains of rats kept under strictly regulated conditions of lighting it was found possible to predict with about 90 per cent accuracy the length of the next cycle in any particular rat Also it was found that under these conditions ovulation occurs between 1–2 a m in the morning Using these rats with regular 5 day cycles Everett (1948) showed that it was possible to shorten the cycle to 4 days (that is to advance ovulation by 24 hours) by injections of progesterone on the third day of dioestrus or of oestrogen on the second day of dioestrus Further work by Everett and Sawyer (1949) showed that the progesterone stimulus to early ovulation may be blocked by dibenamine or atropine By injecting progesterone on the third day of dioestrus followed by

the cyclical nature of oestrus received general acceptance for many years These workers suggested that the oestrous rhythm was endocrine in origin and depended on a reciprocal influence between the pituitary gland and ovary It was thought that oestrogens directly inhibited the secretion of follicle stimulating hormone by the anterior pituitary gland and excited the secretion of the luteinizing hormone and that progesterone directly in

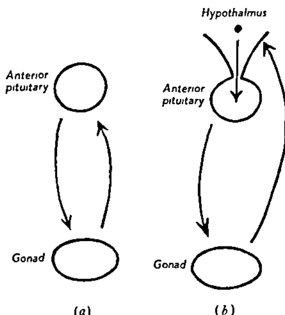


Fig 36 To illustrate the views of (*a*) Moore and Price that the rhythmic nature of reproductive cycles is dependent on the reciprocal reaction between ovary and anterior pituitary and (*b*) Hohlweg and Junkmann that sexual rhythm is dependent upon ovarian hormones affecting the anterior pituitary gland through the intermediation of the hypothalamus

hibited the secretion of luteinizing hormone In this way a self regulating reciprocity between the two glands was thought to be responsible for the cycles of oestrus However Hohlweg and Junkmann (1932) and Hohlweg (1936) noted that anterior pituitary transplants do not develop castration cells after ovariectomy of the host They postulated the existence of a sexual centrum in the hypothalamus a centre which normally stimulates the adenohypophysis to secrete follicle stimulating hormone but

experiments are particularly instructive since the dose necessary to block ovulation is so small that even ataxia in the treated animal may be difficult to recognize Everett and Sawyer draw attention to the similarities between the blocked ovulation due to barbiturate sedation and the state of prolonged oestrus produced in rats by continuous illumination (Browman 1937 Hemmingsen and Krarup 1937) In susceptible animals this latter effect may begin within a matter of days (Everett 1942) Further reversal of oestrus (mating normally occurs during the night in the rat) follows reversal of day night lighting conditions (Hemmingsen and Krarup 1937) All these data are most easily explained on the theory that the spontaneous ovulation and the cycles of oestrus of the rat are controlled neurally It would be of interest if experiments of this type were repeated on other spontaneously ovulating forms such as primates

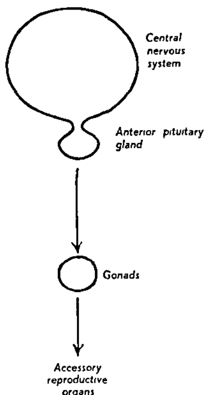


Fig 37 The more important organs involved n the process of sexual maturation

Puberty

The factors underlying the onset of reproductive activity at puberty have been discussed for many years The organs mainly concerned are depicted in Fig 37 It has been known since the work of Foa (1900) that the gonads are able to function in adult fashion long before puberty The ovaries of immature animals transplanted to mature animals may show changes typical of the adult organ likewise ovaries from a mature animal transplanted to the prepubertal become quiescent and atrophy Also the

atropine blockade at different intervals it was found that regardless of how early progesterone was administered activation of the hypophysis did not begin till 2 p m or later, becoming complete in about half the animals before 4–5 p m That is to say the stimulus for release of the luteinizing hormone induced by progesterone occurred at a definite time of the day which was not related to the time of progesterone administration Everett Sawyer and Markee (1949) also showed that the normal spontaneous ovulation of the 4 day cyclic rat could be blocked in a large proportion of cases by dibenamine given before 2 p m on the day of pro oestrus and in all animals by atropine given during the same time interval When either drug was injected after 4 p m on the day of pro oestrus ovulation occurred normally during the following night (except for a few rats treated with dibenamine) From all these experiments it was concluded that the stimulus evoking release of luteinizing hormone from the anterior pituitary gland in either spontaneously ovulating or progesterone stimulated rats occurs at 2–4 p m on the day of pro oestrus This release of luteinizing hormone is followed by ovulation 10–12 hours later that is at 2–4 a m the next morning It is of interest to compare these time intervals with those of the rabbit where coitus stimulates the release of the luteinizing hormone and is followed by ovulation 10 hours later The presence of a chronologically limited stimulus to the release of the luteinizing hormones in the rat and the fact that the stimulus may be blocked by dibenamine or atropine drugs which do not affect the release of luteinizing hormone from the pituitary gland and do not affect the action of this hormone on the ovary strongly suggests the participation of the central nervous system and probably the hypothalamus in the control of ovulation in the rat It may then reasonably be argued that the effect of oestrogens and progesterone on ovulation are also mediated by the nervous system The more recent work of Everett and Sawyer (1950) lends further support for these views They found that nembutal and other barbiturates prevent the ovulatory activation of the hypophysis if administered at 2 p m on the day of pro oestrus If nembutal treatment was repeated at the same time on 2 or 3 successive days persistence of the graafian follicles occurred If treatment was omitted on any of these 3 days or postponed until after 4 p m ovulation occurred during the next night These

resulting in premature puberty in young children again demonstrates the capacity of the human pituitary and gonad to function before the normal age of puberty and also indicates the neural origin of the changes of puberty No attempts seem to have been made to reproduce this condition experimentally but it would be of much interest to see whether lesions in the posterior tuberal or mammillary regions of the hypothalamus or prolonged electrical stimulation of the same areas resulted in premature puberty in other mammals

Before puberty during the quiescent stage of a breeding season and after the menopause there appears to be a lack of some stimulus otherwise transmitted from the hypothalamus to the adenohypophysis

Sexual differentiation of the hypothalmus

The hypophysis of the male animal appears to secrete gonadotrophins at a steady rate during the active period of the breeding season and does not show the rhythmic fluctuations typical of the female cycles This does not seem to be due entirely to the presence of the testis for ovaries transplanted into a castrate male fail to form corpora lutea or to show cyclic activity (Goodman 1934 Pfeiffer 1936 Deanesly 1938 see however Katsh 1950) Pfeiffer (1936 1937) believed that this was due to the anterior pituitary gland becoming sexually differentiated during infancy under the early action of androgens It is now clear that this cannot be the case for anterior pituitary tissue obtained from adult male rats transplanted under the median eminence of the hypothalamus of hypophysectomized adult female rats where it became revascularized by the pituitary portal vessels has been found effective in maintaining normal oestrous cycles and pregnancy (Harris and Jacobsohn 1952) From this observation it would seem that anterior pituitary tissue remains plastic or pluripotential in its functional capacity and that its activity in a male or female animal depends on the stimulus the gland tissue receives from the hypothalamus In view of the fact that Pfeiffer (1936) showed prepubertal testis grafts in female rats produce a constant oestrous state after puberty and that ovaries grafted into adult male rats castrated at birth do undergo cyclic changes it seems likely that some neural structure in the male animal becomes differentiated and fixed in its function under the

new-born human female may show a vicarious menstruation due to the effect of maternal gonadotrophins Thus the ovaries and accessory organs are able to function actively under appropriate conditions long before the age of puberty Following the work of Philip Smith on the gonadotrophic activity of the anterior pituitary gland the view became current that the adenohypophysis was the regulator of pubertal changes However, implantation of the anterior lobes of immature animals into other immature animals may induce gonadal activity and it has been found that the gonadotrophic content of the anterior pituitary may be high long before puberty Robson (1947) has summarized the position Thus the anterior pituitary lobe contains active gonadotrophic hormone before the onset of maturity and the ovary is capable of reacting to this hormone and yet under normal physiological conditions maturity does not occur at that time It seems that a major factor responsible for puberty is an increased rate of release of pituitary gonadotrophin though changes in the sensitivity of the organs to circulating hormone may contribute (Hooker 1942) The cause of the increased gonadotrophin release from the pituitary is unknown but it is likely that a neural (hypothalamic) stimulus via the hypophysial portal vessels may be involved

(*a*) Hypophysectomized female rats which had had pituitary tissue obtained from their own young grafted under the median eminence resumed oestrous cycles within 8–35 days from grafting that is at a time when their litters would have been 12–43 days of age (Harris and Jacobsohn 1952) The anterior pituitary tissue responsible for these oestrous cycles thus showed greatly hastened development for puberty in the rat does not normally occur until about 40–70 days of age This effect was only seen in animals in which the pituitary graft became revascularized by the portal system of vessels It seems unlikely that this result can be due to the fact that a single graft consisted of several (immature) glands

(*b*) The onset of sexual maturation may be accelerated by prolonged electrical stimulation of the uterine cervix in prepubertal rats (Swingle Seay Perlmutt Collins Fedor and Barlow 1951) This may indicate release of gonadotrophin under nervous reflex stimulation

(*c*) The rare but well affirmed cases of hypothalamic tumours

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CHAPTER 5

REGULATION OF ADRENOCORTICOTROPHIC SECRETION FROM THE ANTERIOR PITUITARY GLAND

Since the introduction of the adrenocorticotrophic hormone (A C T H) and cortisone for therapeutic use the investigation of the factors which normally regulate the secretion of A C T H have received much attention

The most important physiological action of A C T H is seen in the effect it exerts on the adrenal cortex Other possible actions of this hormone such as an effect on granulation tissue formation mediated via the ovary (Clayton and Prunty 1951) and an effect on the electrical activity (Torda and Wolff 1952*a*)

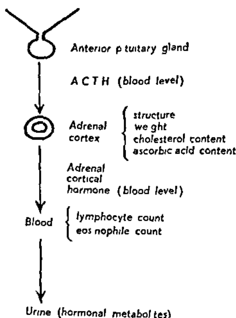


Fig 38 To llustrate the methods used to measure the activity of the pitu tary adren l axis

and acetylcholine synthesizing power (Torda and Wolff 1952*b*) of the brain may represent physiological actions of the hormone independent of adrenal cortical activation but are not germane to the following discussion

In the majority of experiments in which the control of A C T H discharge from the anterior pituitary gland has been investigated one or more of the factors represented diagrammatically in Fig 38 were used as indicating the rate of such discharge The secretion of A C T H may be studied by measuring the variations in the concentration of this hormone in the blood by observing the effects it exerts on the adrenal cortex or the effects produced by the increased adrenal cortical secretion However no one of the methods is entirely satisfactory

(*a*) A method for measuring the amount of A C T H in peripheral blood has recently been devised (see Sydnor and Sayers 1952) Clearly the most direct method of detecting changes in the rate of secretion of the hormone is to measure the varying concentrations of A C T H in pituitary venous blood At the moment this is not possible since pure venous blood from the pituitary gland cannot be obtained Such assays may be performed on peripheral blood but the interpretation of such measurements involves the question of the rate of hormone utilization as well as that of the rate of hormone secretion

(*b*) Different extracts of anterior pituitary tissue have been obtained which are predominantly active either in maintaining the weight of the adrenal gland after hypophysectomy *or* in depleting the ascorbic acid content of the gland (Stack Dunne and Young 1951 Stack Dunne 1953) It is possible that these extracts represent two different hormones secreted by the pituitary gland in which case neither the maintenance of adrenal weight nor depletion of ascorbic acid tests would alone give the complete picture of A C T H release It is however also possible that these two extracts are in a sense biochemical artefacts and are not representative of naturally occurring hormones

(*c*) Biological and chemical methods for measuring the concentration of adrenal cortical secretion in peripheral blood have been devised but are on the whole lengthy and laborious and have rarely been used in the studies discussed below

(*d*) Methods based on adrenal weight or histology are slow or difficult to place on a quantitative basis

(*e*) Methods based on the chemical measurement of adrenal ascorbic acid or adrenal cholesterol content have been frequently used They are quick and simple to perform The role played by ascorbic acid in the adrenal cortex is unknown but it is well established that after an injection of A C T H or after the application of stress to a mouse rat or guinea pig the ascorbic acid content of the adrenal falls This reduction in ascorbic acid is the basis for the Sayers assay method (Sayers, Sayers and Woodbury 1948) for A C T H and under carefully standardized conditions and in practised hands is highly specific and sensitive The fact that the metabolism of ascorbic acid in the adrenal gland varies in different forms (the guinea pig is unable to synthesize this acid A C T H injections or stress produce little ascorbic acid depletion in the adrenal glands of chick duck quail or golden hamster—see Zarrow and Baldini 1952) and the fact that the adrenal gland of the scorbutic guinea pig may still show cholesterol depletion following injection of A C T H at a time when the ascorbic acid content of the gland has practically disappeared (Oesterling and Long 1951) show that there are limitations to the use of this method under certain circumstances The depletion of adrenal cholesterol following injection of A C T H or application of stress has not been studied so closely or so widely used as the ascorbic acid method However the demonstration that the perfused adrenal gland can introduce an oxygen atom at the C 11 position of the cholesterol molecule (Hechter Jacobsen Jeanloz Levy Marshall Pincus and Schenker 1949) has made it probable that cholesterol in the adrenal cortex is a store of precursor for the formation of adrenal steroids and the cholesterol depletion method for measuring A C T H release would therefore seem to be more logically founded than that based on ascorbic acid

(*f*) Methods based on counts made of the circulating lymphocytes and eosinophiles in the blood stream have been widely practised since they are rapid and easy to perform and may be used on the unanaesthetized animal or human Such methods were established by the work of Dougherty and White (1944) and Hills Forsham and Finch (1948) when they showed that injection of A C T H or adrenal corticoids oxygenated at C 11 position resulted in a lymphopenia or eosinopenia Stress stimuli which cause liberation of endogenous A C T H pro

and acetylcholine synthesizing power (Torda and Wolff, 1952*b*) of the brain may represent physiological actions of the hormone independent of adrenal cortical activation but are not germane to the following discussion

In the majority of experiments, in which the control of A C T H discharge from the anterior pituitary gland has been investigated, one or more of the factors represented diagrammatically in Fig 38 were used as indicating the rate of such discharge The *secretion of A C T H may be studied by measuring* the variations in the concentration of this hormone in the blood by observing the effects it exerts on the adrenal cortex or the effects produced by the increased adrenal cortical secretion However no one of the methods is entirely satisfactory

(*a*) A method for measuring the amount of A C T H in peripheral blood has recently been devised (see Sydnor and Sayers 1952) Clearly the most direct method of detecting changes in the rate of secretion of the hormone is to measure the varying concentrations of A C T H in pituitary venous blood At the moment this is not possible since pure venous blood from the pituitary gland cannot be obtained Such assays may be performed on peripheral blood but the interpretation of such measurements involves the question of the rate of hormone utilization as well as that of the rate of hormone secretion

(*b*) Different extracts of anterior pituitary tissue have been obtained which are predominantly active either in maintaining the weight of the adrenal gland after hypophysectomy *or* in depleting the ascorbic acid content of the gland (Stack Dunne and Young 1951 Stack Dunne, 1953) It is possible that these extracts represent two different hormones secreted by the pituitary gland in which case neither the maintenance of adrenal *weight nor depletion of ascorbic acid tests would alone* give the complete picture of A C T H release It is however also possible that these two extracts are in a sense biochemical artefacts and are not representative of naturally occurring hormones

(*c*) Biological and chemical methods for measuring the concentration of adrenal cortical secretion in peripheral blood have been devised, but are on the whole lengthy and laborious and have rarely been used in the studies discussed below

(*d*) Methods based on adrenal weight or histology are slow or *difficult to place on a quantitative basis*

(*e*) Methods based on the chemical measurement of adrenal ascorbic acid or adrenal cholesterol content have been frequently used They are quick and simple to perform The role played by ascorbic acid in the adrenal cortex is unknown, but it is well established that after an injection of A C T H, or after the application of stress to a mouse rat or guinea pig the ascorbic acid content of the adrenal falls This reduction in ascorbic acid is the basis for the Sayers assay method (Sayers Sayers and Woodbury 1948) for A C T H and under carefully standardized conditions and in practised hands is highly specific and sensitive The fact that the metabolism of ascorbic acid in the adrenal gland varies in different forms (the guinea pig is unable to synthesize this acid A C T H injections or stress produce little ascorbic acid depletion in the adrenal glands of chick duck quail or golden hamster—see Zarrow and Baldini 1952) and the fact that the adrenal gland of the scorbutic guinea pig may still show choles terol depletion following injection of A C T H at a time when the ascorbic acid content of the gland has practically disappeared (Oesterling and Long 1951) show that there are limitations to the use of this method under certain circumstances The depletion of adrenal cholesterol following injection of A C T H or applica tion of stress has not been studied so closely or so widely used as the ascorbic acid method However the demonstration that the perfused adrenal gland can introduce an oxygen atom at the C 11 position of the cholesterol molecule (Hechter Jacobsen Jeanloz Levy Marshall Pincus and Schenker 1949) has made it probable that cholesterol in the adrenal cortex is a store of precursor for the formation of adrenal steroids and the choles terol depletion method for measuring A C T H release would therefore seem to be more logically founded than that based on ascorbic acid

(*f*) Methods based on counts made of the circulating lympho cytes and eosinophiles in the blood stream have been widely practised since they are rapid and easy to perform and may be used on the unanaesthetized animal or human Such methods were established by the work of Dougherty and White (1944) and Hills Forsham and Finch (1948) when they showed that in jection of A C T H or adrenal corticoids oxygenated at C 11 position resulted in a lymphopenia or eosinopenia Stress stimuli which cause liberation of endogenous A C T H pro-

duce the same result However in employing these criteria of A C T H release it is necessary to consider (1) the animal used (for example rabbits have a high proportion of lymphocytes and pseudo eosinophiles and are thus suitable for studies of lympho penic response but not eosinopenic) (2) the anaesthetic used if any (urethane has a direct action on lymphocytes) and (3) to remember that factors other than the A C T H content of the blood may result in lymphopenia or eosinopenia Hungerford (1949) has described a lymphopenia following injection of large (about 140 μg) doses of adrenaline into hypophysectomized but not adrenalectomized rats The eosinopenic response is perhaps more open to criticism from this point of view for Speirs and Meyer (1949) report that eosinopenia follows injection of adrena line (5 μg) or stress in hypophysectomized mice Also it has been reported that large doses of adrenaline will produce an eosinopenia in patients with Addison s disease or in the human after bilateral adrenalectomy (Thorn 1952) It seems likely, at the moment that injection of adrenaline may produce an eosino penia by potentiating the action of (exogenous or endogenous) adrenal corticoids already in the blood stream as well as by causing A C T H release from the pituitary However it may be mentioned that Speirs (1953) has with suitable precautions developed a method of assay of A C T H based on the eosino penic response of intact or hypophysectomized mice and that Long and his colleagues (see Long 1952) have obtained an ex cellent correlation in the rat between the fall in circulating eosinophiles and the reduction of adrenal ascorbic acid and be tween the fall in lymphocytes and the decline in adrenal choles terol

For further details concerning methods of measuring or detecting A C T H secretion reference may be made to the review of Sayers (1950)

Theories Concerning the Regulation of A C T H Secretion

The activity of the adrenal cortex is dependent on several factors of which the most important appears to be the secretion of A C T H by the adenohypophysis That this is not the only factor is shown by the finding that hypophysectomy does not result in complete cessation of activity by the adrenal cortex life

is maintained better after hypophysectomy than adrenalectomy Few investigations have been made of the extrahypophysial factors responsible for adrenal cortical activity Vogt (1951a) has shown that the isolated adrenal gland of the dog perfused with a Dale Schuster pump continues to secrete cortical hormone at a steady rate for a period of up to one hour and a half The rate of secretion of this isolated gland may be increased by adding A C T H to the perfusing blood but it may also be increased by adding adenosinetriphosphate creatine phosphate large doses of histamine or by increasing the potassium/sodium ratio of the plasma

Bush (1951) has suggested dividing the activity of the adrenal cortex into steady state function (which may be independent of the pituitary) and stress function (dependent on the pituitary adrenal relationship) There is no doubt that the increased secretion of the adrenal cortex observed under a wide variety of conditions of stress (thermal bacterial traumatic toxic emotional etc) is due to an increased rate of liberation of pituitary A C T H elicited by the stress However since animals kept under optimum quiescent conditions possess more adrenal cortical tissue than hypophysectomized animals this classification would indicate that the steady state' or non stressed state is only theoretically possible This is likely and it is of interest that the position of the adrenal cortex would then appear similar to that of the adrenal medulla at the time of the controversy between the schools maintaining continuous or emergency secretion of adrenaline On these lines the problem of classifying adrenal activity becomes mainly one of semantics regarding the meaning of terms such as steady state emergency or stress It is perhaps not without significance that one of the simplest ways of describing stress stimuli (to include also states of emotional stress) is stimuli which in the normal animal excite increased activity of the sympathetic nervous system and adrenal medulla

In the last few years discussion of the factors regulating A C T H secretion have been concerned in the main with the factors regulating the increased secretion of A C T H under conditions of stress Three views have been put forward not necessarily incompatible concerning this regulation (1) A C T H secretion is regulated by the systemic blood level of adrenaline,

(2) A C T H secretion is regulated by the systemic blood level of the adrenal cortical hormones (3) A C T H secretion is controlled by the hypothalamus acting through the hypophysial portal vessels These views will now be discussed

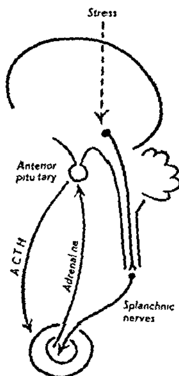
(A) Systemic blood level of adrenaline

In 1943 Dr Marthe Vogt described for the first time the very high rate of secretion of the adrenal cortex She found the concentration of hormone in venous blood from the gland to be surprisingly high as compared with the small amount of hormone stored in the adrenal at any one time In order to estimate the amount of cortical hormone in samples of adrenal venous blood she used the Selye Schenker method which measures the life maintaining power of the substance as observed after injection into young adrenalectomized rats exposed to low temperature In the following year Vogt (1944) described the increased output of cortical hormones that occurs following the intravenous infusion of adrenaline (6–8 μg/kg) into the eviscerated dog or cat The output of hormone was increased several times by this procedure and was shown to be independent of the pressor response of adrenaline and of variation in blood flow through the adrenal At first it was thought likely that adrenaline acts directly on the cells of the adrenal cortex since increased activity of this gland was observed to occur a few minutes after starting adrenaline infusion However the latent period between the release of A C T H and increased activity of the adrenal cortex was later (Vogt 1947) found to be of the same order of time The following findings now make it clear that the major action of adrenaline in eliciting adrenal cortical activation is mediated in some way via the pituitary gland

(i) Hypophysectomy prevents the depletion in adrenal cholesterol and ascorbic acid that normally follows injection of adrenaline (Long and Fry 1945) and prevents the accumulation of lipins and weight increases which normally follow chronic administration (1–3 weeks) of adrenaline (Vogt 1945)

(ii) The isolated perfused adrenal cortex is not stimulated to increased activity by addition of adrenaline to the perfusing blood (Hechter 1949 Vogt 1951*a*) but is stimulated by the addition of A C T H

The effect of other sympathomimetic amines in causing discharge of pituitary A C T H have been studied and it is generally agreed that adrenaline is one of the most potent in this respect For example Nasmyth (1950) found that the relationship between doses of L-adrenaline L noradrenaline and p sympatol in causing equal pressor responses was 1 0 4 200 whereas the relationship of doses causing equal falls in the ascorbic acid content of the rat adrenal gland was 1 45 1200 These results have been confirmed by Jarrett (1951) who also found that dl isopropyl noradrenaline (isoprenaline) is as potent in depleting ascorbic acid in the adrenal gland of the rat as l adrenaline in spite of the fact that it consists of two isomers one of which may be inactive



F g 39 Diagram to illustrate the views of Long and his colleagues
Conditions of stress are bel eved to activate a neural mechanism in the posterior hypothalamus which in turn exc tes secretion of adrenaline The inc eased blood level of adrena l ne is thought to be a major factor in the immediate release of A C T H following stress

In 1947 Long argued that since stresses of various types are known to result in secretion of adrenaline and A C T H and since adrenaline will stimulate release of A C T H then in all probability adrenaline is part of a physiological mechanism regulating A C T H secretion in response to stress This view may be expressed diagrammatically as in Fig 39 The data on which this view is based are given by Gershberg Fry Brobeck and Long (1950) and McDermott Fry Brobeck and Long (1950*a*) These workers showed that infusions of small physiological doses of adrenaline in rats resulted in A C T H secretion as demonstrated by a fall in adrenal ascorbic acid or adrenal

cholesterol The time of infusion was found to be [illegible] whereas the intramuscular infusion of 0.5 μg of adr[illegible] period of an hour was followed by a significant de[illegible] adrenal ascorbic acid the infusion of the same quantity [illegible] had no effect Various stress procedures such as c[illegible] sensory nerve stimulation haemorrhage and lapar[illegible] also found to result in reduction of adrenal ascorbic [illegible] normal but not in the hypophysectomized rat In o[illegible] there is a causal relationship between adrenaline and [illegible] secretion in response to stress Long and his colle[illegible] investigated the effects of stresses after demedulla[illegible] adrenal glands and after interrupting the neural p[illegible] responsible for adrenaline secretion by placing lesions in diencephalon and by cutting the spinal cord at the level of third thoracic vertebra In these studies the criterion of ACT[illegible] secretion was an eosinopenic response It was found [illegible] adrenal demedullated rats no longer responded to mild [illegible] stimuli (stimulation of an afferent nerve trunk for 15 min) [illegible] such rats still showed a marked response to more severe s[illegible] (fasting plus exposure to cold for 4 hours haemorrh[illegible] laparotomy) Section of the spinal cord was found to abo[illegible] A C T H secretion in response to a mild stress (injection [illegible] hypertonic saline beneath the skin of the scalp) but a mar[illegible] response was still obtained following a more severe stress (co[illegible] Likewise rats with diencephalic lesions responded to laparot[illegible] by release of A C T H

A study of the time relations of these responses showed that normal rats gave a marked response (eosinopenia) within o[illegible] hour of the stress the response being maximal at the fourth hour The rats in which adrenaline secretion was prevented showed n[illegible] response at one hour but a normal response at the fourth hou[r] For this reason Long and his colleagues suggested that a double mechanism underlies the increased secretion of A C T H fol lowing stress (a) a quick autonomic reaction depending on reflex secretion of adrenaline which in turn activates the a[illegible] pituitary and (b) a slower metabolic mechanism, probably dependent on the lowered blood level of adrenal cortical hormo[ne] [illegible] which is not abolished by adrenal [illegible]

[illegible]

[illegible] action of adrenaline in

effecting A C T H secretion McDermott Fry Brobeck and Long (1950*b*) transplanted anterior pituitary tissue to the anterior chamber of the eye in hypophysectomized rats In these animals eosinopenic responses were observed to follow subcutaneous injection of adrenaline or injection of very small doses of adrenaline into the eye containing the graft but not into the normal eye As a final experiment on these animals the eye bearing the graft was removed and it was then found that subcutaneous injection of adrenaline no longer resulted in an eosinopenia The conclusion drawn from these experiments was that adrenaline causes A C T H secretion by a direct action on anterior pituitary cells It is perhaps significant as reported in Chapter 3 that these pituitary transplants did not prevent partial adrenal atrophy or complete atrophy of the testes and did not maintain body growth Similar results concerning the lack of maintenance of the adrenal gland testes and body growth were obtained by Fortier (1951 1952) who also studied the A C T H potency of pituitary transplants into the anterior chamber of the eye of rats Fortier divided his stressing stimuli into two groups systemic (adrenaline cold histamine) and neurotropic (sound immobilization) The rats bearing transplants and normal controls were stimulated at weekly intervals and the fall of the circulating blood eosinophiles used as an index of A C T H release Adrenaline cold and histamine brought about a definite eosinopenia in both normal and grafted animals Sound and immobilization induced a marked fall of the eosinophiles in the intact but none in the grafted animals In a complementary experiment direct stimulation of the hypophysial transplant in the eye was attempted by localized cooling of the eye and by subconjunctival injection of adrenaline histamine and dibenamine Two of these procedures injection of adrenaline and histamine resulted in an eosinopenia From these experiments Fortier concluded that neurotropic stress stimuli activate A C T H release by means of hypothalamo hypophysial connections whereas systemic stress stimuli may act directly on anterior pituitary cells The fact that locally applied histamine as well as adrenaline stimulated A C T H secretion by the pituitary transplants throws serious doubt on the specific nature of the response of the pituitary for adrenaline

The view upheld by Long and his co workers that adrenaline secretion is part of a physiological mechanism underlying secre

cholesterol The time of infusion was found to be important for whereas the intramuscular infusion of 0 5 μg of adrenaline in a period of an hour was followed by a significant decrease of adrenal ascorbic acid the infusion of the same quantity in 15 min had no effect Various stress procedures such as cold exposure sensory nerve stimulation haemorrhage and laparotomy were also found to result in reduction of adrenal ascorbic acid in the normal but not in the hypophysectomized rat In order to see if there is a causal relationship between adrenaline and A C T H secretion in response to stress Long and his colleagues have investigated the effects of stresses after demedullation of the adrenal glands and after interrupting the neural pathway responsible for adrenaline secretion by placing lesions in the diencephalon and by cutting the spinal cord at the level of the third thoracic vertebra In these studies the criterion of A C T H secretion was an eosinopenic response It was found that adrenal demedullated rats no longer responded to mild stress stimuli (stimulation of an afferent nerve trunk for 15 min) but such rats still showed a marked response to more severe stress (fasting plus exposure to cold for 4 hours haemorrhage or laparotomy) Section of the spinal cord was found to abolish A C T H secretion in response to a mild stress (injection of hypertonic saline beneath the skin of the scalp) but a marked response was still obtained following a more severe stress (cold) Likewise rats with diencephalic lesions responded to laparotomy by release of A C T H

A study of the time relations of these responses showed that normal rats gave a marked response (eosinopenia) within one hour of the stress the response being maximal at the fourth hour The rats in which adrenaline secretion was prevented showed no response at one hour but a normal response at the fourth hour For this reason Long and his colleagues suggested that a double mechanism underlies the increased secretion of A C T H following stress (*a*) a quick autonomic reaction depending on reflex secretion of adrenaline which in turn activates the anterior pituitary and (*b*) a slower metabolic mechanism probably dependent on the lowered blood level of adrenal cortical hormone under conditions of stress and which is not abolished by adrenal demedullation or denervation

In an attempt to localize the site of action of adrenaline in

10 sec following an intravenous injection of histamine seems to exclude the participation of adrenaline in this release A single rapid intravenous injection of adrenaline is ineffective as a stimulus to A C T H release (Colfer de Groot and Harris 1950, Gray and Munson, 1951), and as mentioned above Long et al found intramuscular infusions of adrenaline were also ineffective unless continued for more than 15 minutes It is difficult to uphold the claim that adrenaline secretion is responsible for the rapid release of A C T H following stress if certain stresses (intravenous injection of histamine) cause A C T H secretion more rapidly than intravenous injection of adrenaline Also the time periods given by Gray and Munson would not be sufficient for histamine to be carried to and to stimulate some neural mechanism causing adrenaline secretion and for the adrenaline so released to be circulated to the pituitary It may be mentioned in this connection that Munson and Greep (1951) found intraperitoneal injection of histamine but not adrenaline produced a marked depletion of adrenal ascorbic acid within 15 minutes from injection The most direct approach to this problem has been recently reported by Vogt (1952) who assayed the plasma level of adrenaline in rats under different circumstances Previous work by Vogt (1951*b*) mentioned above had shown that emotional stress liberation of A C T H in the rat was reduced but not abolished by adrenal demedullation The possibility existed that the residual release of A C T H by emotion was due to liberation of adrenaline from some source other than the adrenal However Vogt assayed the plasma adrenaline of adrenal demedullated rats exposed to a stress sufficient to cause a fall in adrenal ascorbic acid and found no adrenaline (<0 5 mμg/ml) in the plasma of these animals Intramuscular injection of adrenaline in amounts hardly sufficient to cause a fall in adrenal ascorbic acid produced concentrations of adrenaline in arterial plasma of 1–4 5 mμg/ml It was possible to say then that the release of A C T H in the group of rats exposed to stress was in no way dependent on the blood level of adrenaline even though the speed of release of A C T H is so fast that a significant fall in adrenal ascorbic acid occurred within approximately 10 minutes Vogt suggests this rapid release is best accounted for by an action of the stress stimulus mediated through the central nervous system

tion of A C T H in response to conditions of stress is open to question

(i) As discussed by Sayers (1950) completely sympathectomized animals may resist many forms of stress almost as well as do intact animals and certainly more efficiently than adrenalectomized animals

(ii) The claim of Long et al that mild stress stimuli no longer effect A C T H liberation in the absence of adrenaline secretion has not been confirmed Colfer de Groot and Harris (1950) observed that the lymphopenic response which followed a mild pain stimulus in rabbits was not altered by adrenal denervation and Vogt (1951*b*) found that emotional stress stimuli still produce a fall in adrenal ascorbic acid after adrenal demedullation in rats It is difficult to compare ' mild stress stimuli as applied in different laboratories but the stress used by Vogt (passage of a rectal thermometer) would appear to be less traumatic than that used by Long et al (stimulation of the sciatic nerve for 15 min)

(iii) The claim of Long et al that adrenaline secretion is a necessity for the quick one hour response following stress stimuli has not been substantiated Vogt (1951*b*) found that the fall in adrenal ascorbic acid that followed emotional stress stimuli in adrenal demedullated rats though not so large as in normal rats did occur within the course of one hour Gordon (1950*a*) has likewise found that insulin histamine and exposure to cold reduce the ascorbic acid content of the demedullated rat adrenal within one hour Similarly Hodges (1953) reported the time relations of the stress induced changes in adrenal ascorbic acid concentration in both adrenal demedullated and normal rats to be similar These observations however do not eliminate the possibility that the adrenal medulla normally plays a role in the response to stress but only that it does not play a necessary role To carry the investigation further Hodges studied the amount of desoxycorticosterone acetate required to block the stress response in normal and adrenal demedullated rats He found that the necessary dose was of the same order in the two groups of rats a result which indicates that the final stimulus to pituitary secretion of A C T H following stress is as potent in the absence of the adrenal medulla as in the normal animal

(iv) The recent work of Gray and Munson (1951) demonstrating release of A C T H by the pituitary within 3 5 or at most

1938) Other workers have found that chronic administration of desoxycorticosterone acetate also results in adrenal atrophy and will inhibit the hypertrophy of the adrenals which normally fol lows exposure to a variety of stress In more acute experiments Sayers and Sayers (1947) showed that the fall in adrenal ascorbic acid which normally follows the application of cold or heat or the injection of typhoid toxin adrenaline or histamine can be

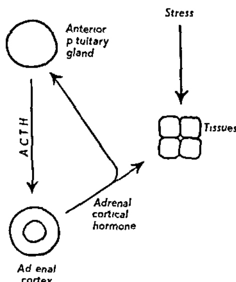


Fig 40 D agram illustrating the view of Sayers that the secretion of A C T H by the anterior pituit ry gland s regulated by the blood level of adrenal cortical hormones

Stress is thought to act directly on the tissues increasing the tissue utilization of the adrenal cortical hormones so that the level of these substances in the blood supply ng the pitu tary gland is lo vered the eby incre s ng the output of A C T H

prevented by the pretreatment of the animal with adrenal cor tical extract or crystalline cortical steroids Since the effect of injected A C T H is not modified by administration of adrenal cortical extracts they concluded that the cortical substances act by inhibiting the release of A C T H from the pituitary gland Sayers and Sayers (1948) also found a quantitative relationship to exist between the dose of cortical steroid administered and the degree of inhibition exerted on A C T H secretion For example

In summary there is little doubt that a raised blood level of adrenaline within physiological limits, is capable of eliciting an increase in the rate of secretion of A C T H and that the removal of the adrenal medulla results in diminution of A C T H liberated in response to mild stress stimuli However this adrenaline mechanism would not appear to be rapid enough to account for the facts now known regarding the speed of A C T H release It appears to be a system whereby an already increased secretion of A C T H, due to stress stimuli *acting through the* central nervous system is reinforced

(B) Systemic blood level of adrenal cortical hormones

It has long been known that a reciprocal relationship holds between the concentration of ovarian and gonadotrophic hormones in the blood stream Administration of oestrogens results in diminution of gonadotrophic secretion and ovarian atrophy whilst the ovarian atrophy which occurs at the menopause results in increased urinary excretion of gonadotrophins It is now known that a similar type of relationship exists between the adrenal cortex and pituitary secretion of adrenocorticotrophin Administration of adrenal cortical extracts results in diminished secretion of A C T H and adrenal atrophy whilst exposure of an animal to mild or moderate degrees of stress after injection of adrenal cortical extracts does not result in the usual increased secretion of A C T H These facts have been utilized by Sayers (see Sayers 1950) in support of the view that A C T H secretion is regulated by the blood level of the adrenal cortical hormone Under conditions of stress it is suggested that the peripheral tissues utilize adrenal cortical hormones more rapidly that the blood content of the hormone falls in consequence and this in turn directly stimulates anterior pituitary tissue to secrete increased amounts of A C T H This mechanism is depicted diagrammatically in Fig 40 According to this view then the anterior pituitary/adrenal cortex forms a self regulating system the balance of which is broken mainly by variations in activity of the peripheral tissues

There can be no doubt of the validity of many of the experimental observations upon which this theory is founded Administration of adrenal cortical hormone over a period of days was shown to cause adrenal atrophy (Ingle, Higgins and Kendall

1938) Other workers have found that chronic administration of desoxycorticosterone acetate also results in adrenal atrophy and will inhibit the hypertrophy of the adrenals which normally follows exposure to a variety of stress In more acute experiments Sayers and Sayers (1947) showed that the fall in adrenal ascorbic acid which normally follows the application of cold or heat or the injection of typhoid toxin adrenaline or histamine can be

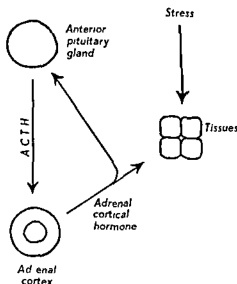


Fig 40 Diagram illustrat ng the view of Sayers that the secretion of A C T H by the anterior pituitary gland s regulated by the blood level of adrenal cortical hormones

Stress is thought to act directly on the ti sues increasing the tissue utilization of the adrenal cort cal hormones so that the level of these substances in the blood supplying the pitu tary gland is lowered thereby increas ng the output of A C T H

prevented by the pretreatment of the animal with adrenal cortical extract or crystalline cortical steroids Since the effect of injected A C T H is not modified by administration of adrenal cortical extracts they concluded that the cortical substances act by inhibiting the release of A C T H from the pituitary gland Sayers and Sayers (1948) also found a quantitative relationship to exist between the dose of cortical steroid administered and the degree of inhibition exerted on A C T H secretion For example

larger amounts of adrenal steroid were required to block the secretion of A C T H in response to a moderate stress (injection of 0 5 mg histamine per 100 g of body weight) than to a milder stress (injection of 0 25 mg histamine per 100 g of body weight) in the rat Long and his co workers have confirmed many of these findings Administration of adrenal cortical extract pre vented the usual fall in adrenal cholesterol and ascorbic acid of rats exposed to cold or subjected to unilateral adrenalectomy (Long 1947) and reduced or prevented the fall in adrenal ascorbic acid following injection of insulin or adrenaline (Gershberg Fry Brobeck and Long 1950)

It seems clear then that the mechanism proposed by Sayers plays some part in regulating A C T H discharge from the anterior pituitary gland but there are many facts that cannot be explained on this view alone

1 Anterior pituitary tissue transplanted to a distant site in the body of hypophysectomized animals does not maintain the adrenal gland in a normal state If the only factor responsible for regulating A C T H discharge was the blood level of adrenal cortical hormone then a well vascularized transplant should maintain a normally active adrenal cortex if placed in regions remote from the sella turcica This is not so for as Cheng Sayers Goodman and Swinyard (1949) found such pituitary transplants resulted in most cases in adrenal glands that were no larger than those of the hypophysectomized control animals

2 Little is known regarding the utilization of adrenal cortical hormone by the peripheral tissues and the few facts which are available do not support the general view of Sayers Nelson (1952) has developed a method for determining the 17 hydroxycortico steroid level in peripheral blood samples Examination of a large series of arterial and venous blood samples obtained simultane ously failed to reveal any arterial venous difference in concentra tions of 17 hydroxycorticosteroid A small number of similar samples obtained from traumatized or diseased limbs also failed to show any arterial venous difference in the steroid level It may be argued however that the method of assay was not sen sitive to detect differences that were of physiological importance More difficult to explain is the fact that the blood level of 17 hydroxycorticosteroid rises after shock therapy in mental cases and that the rise is immediate (within 5 minutes) without any

sign of a preliminary fall These observations are reinforced by those of Weichselbaum Margraf and Elman (1953) that operative trauma in the human results in a raised blood corticoid concentration and of Hardy Richardson and Dohan (1953) that major operations in man are followed by markedly increased amounts of 17 hydroxycorticoids in the urine Franksson Gemzell and v Euler (1954) made a detailed study of the 17 hydroxycorticosteroid blood levels and the catechol amine output in the urine in a group of patients undergoing major operations or that had suffered traumatic injuries They found that all cases showed an elevated blood level of corticosteroids immediately following operation and for the ensuing 38 hours Their data showed that this rise could occur without any accompanying secretion of adrenaline These results are difficult to explain on the Sayers s hypothesis or on the adrenaline theory of Long

3 Blood from the aorta eventually supplies both the peripheral tissues and the pituitary gland Therefore under conditions of prolonged stress the peripheral tissues could not receive an increased supply of adrenal cortical hormones for the pituitary gland would also receive the same increased supply which would in turn reduce the output of A C T H and so of adrenal cortical hormones That the blood level of adrenal cortical hormones may remain elevated under such conditions is rendered very probable by the fact that the urinary excretion of cortical hormones may be markedly elevated for three weeks or longer after trauma (Venning and Browne 1949)

4 The time relations found by Gray and Munson (1951) mentioned above that A C T H secretion elicited by intravenous injection of histamine starts within 3–10 sec is very difficult to understand on this view

5 Bush Eik Nes and Samuels (1953) working on dogs found that dilution of the carotid blood by intra arterial infusion with warm saline resulted in a fall in pituitary A C T H discharge instead of a rise

6 The fact that injection of adrenal cortical extract fails to prevent the release of A C T H that follows certain stresse (infection with *B tularense* in rats Pinchot Close and Long 1949 fasting in guinea pigs D Angelo Gordon and Charipper 1948 injection of large doses of histamine in rats Sayers and

Sayers, 1947 exposure to cold injection of histamine trauma and audiogenic stimulation Fortier Yrarrazaval and Selye 1951) argues against this humoral regulation of A C T H being the only mechanism involved

7 The recent observations of Sydnor and Sayers (see Sayers 1954) on the A C T H levels in the blood of adrenalectomized rats affords the most convincing proof that under certain circum stances the stimulus of stress to increase A C T H secretion involves neither the blood level of the adrenal cortical hormone nor adrenal medullary hormone Sydnor and Sayers found that the already high blood level of A C T H of the adrenalectomized animal could be raised still higher by stress

In summary it may be stated that the evidence is incontrovert ible that the blood level of adrenal cortical hormone influences the rate of secretion of A C T H in the same way that the blood level of thyroxine affects T S H secretion and of oestrogen affects gonadotrophic secretion Probably these reactions form neither the main stimulus to the secretion of the pituitary trophic hormones under optimum conditions nor the stimulus to changes in secretory rate under conditions of emergency or stress It seems more likely that this mechanism normally acts to set a more constant base line level of secretion against the background of which other factors adjust pituitary activity according to the needs of the organism

(C) Hypothalamic control

The simple experiment of removing the pituitary gland from the sella turcica and transplanting it to another site in the body demonstrates clearly the fundamental role played by the hypo thalamus in maintaining anterior pituitary activity If the trans planted tissue is placed in a site remote from the sella turcica partial or complete atrophy of the adrenal cortex ovaries and thyroid gland occurs (see Chapter 3) If however the trans planted tissue is placed in the vicinity of the hypothalamus and pituitary stalk then regeneration of the hypophysial portal vessels occurs and anterior pituitary function returns as shown by the maintenance of normal reproductive organs adrenal and thyroid glands (Harris and Jacobsohn 1952)

In 1950 de Groot and Harris suggested that some neural mechanism in the hypothalamus is largely responsible for main

taining and regulating the secretion of A C T H by the adenohypophysis They postulated that hypothalamic nerve fibres liberate some chemical transmitter into the hypophysial portal vessels which is carried to the anterior pituitary gland to exert a specific influence over the activity of the gland This view is expressed diagrammatically in Fig 41

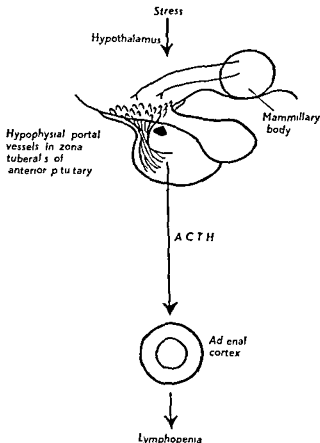


Fig 41 Diagram representing the view that the secretion of A C T H is controlled by the hypothalamus through the mediation of the portal vessels Electrical stimulation of the hypothalamus results in a lymphopenia whereas bilateral lesions placed at this site prevent the lymphopenia that normally follows emotional stress Electrical stimulation of the zona tuberalis does not excite a lymphopenia though lesions here abolish the response that normally follows emotional stress

The experimental basis for the suggestion is as follows Colfer de Groot and Harris (1950) found that the rabbit reacted as other forms in that emotional stress stimuli such as restraint or subcutaneous faradism evoke a lymphopenia Hypophysectomy abolished this response to stress though injection of A C T H was still followed by a lymphopenia in the hypophysectomized animal There was then available for investigation a reaction which could be indicated emotional stress ⟶ anterior pituitary gland ⟶ adrenal cortex ⟶ lymphopenia To see whether A C T H secretion could be evoked by electrical stimulation of the hypothalamus de Groot and Harris (1950) used the remote control technique with the primary coil enlarged to surround the cage containing the animal (see Chapter 4) Thus the animal was not anaesthetized and was not subjected to concurrent emotional stress during the periods of stimulation It was found that stimulation of the posterior region of the tuber cinereum or mammillary body resulted in a lymphopenia that was *similar in time relation and magnitude to that following an* emotional stress stimulus or intravenous injection of an appropriate dose of A C T H Stimulation of other regions in the hypothalamus (including the supraopticohypophysial tract in the anterior part of the median eminence) and pituitary gland (pars and zona tuberalis pars distalis pars intermedia and infundibular stem) did not elicit the response Results which are in the *main similar to these have been obtained by Hume and Wittenstein* (1950) by remote control stimulation of the hypothalamus of the unanaesthetized dog (see also Hume 1952) Using the eosinophile count as an indication of A C T H release they reported that stimulation of the anterior hypothalamus resulted in A C T H secretion in the intact or completely sympathectomized dog This latter observation eliminated the possibility that the stimulus was acting by exciting the secretion of adrenaline More recently Hume (1953) has reported that the stimulation of the posterior tuber cinereum of the hypothalamus of dogs also results in an eosinopenia Porter (1953 and 1954) working with the cat and monkey found that stimulation of the mammillary bodies or posterior tuber cinereum (but not of the anterior hypothalamus) results in an eosinopenia There can be little doubt that the posterior hypothalamus is in some way functionally related to the secretion of A C T H

The effect of hypothalamic lesions on the reflex activation of A C T H secretion in response to stress stimuli have now been studied by several groups de Groot and Harris (1950) found that bilateral electrolytic lesions placed in the mammillary body or posterior part of the tuber cinereum reduced or abolished the lymphopenia produced by emotional stress Single midline lesions placed in various parts of the pituitary gland had no effect on the lymphopenic response to such stress with the exception of those lesions that destroyed the zona tuberalis at the anterior pole of the gland (the part of the gland traversed by the portal vessels in passing from the tuber cinereum to the main mass of the anterior pituitary) This latter finding had especial interest in view of the fact that electrical stimulation of the zona tuberalis had failed to give any sign of A C T H release The conclusion drawn was that stimulation or lesions in the hypothalamus were affecting nerve fibres whereas in the zona tuberalis the effective structures were the hypophysial portal vessels which may be interrupted by an electrolytic lesion but are not affected by electrical stimulation Hume and Wittenstein (1950) Hume (1952 1953) and Long and his co workers (see Brobeck 1952) have also reported that hypothalamic lesions diminish or abolish A C T H secretion in response to stress No detailed publication of the findings of Long et al have yet appeared though the results have been interpreted by these workers as due to interference with the neural mechanism concerned with adrenaline secretion Working on dogs Hume and Wittenstein (1950) found paramedian lesions in the anterior hypothalamus and at the junction of the middle and posterior hypothalamus abolished the stress release of A C T H though Hume (1952) later reported that the anterior part of the median eminence was the most effective location In some of his animals in which the stress response was abolished the lesions also extended into the posterior tuber cinereum and mammillary bodies (see Fig 5 Hume 1953) Ganong and Hume (1954) have also found that lesions of the median eminence in dogs abolish the adrenal hypertrophy that normally follows trauma or unilateral adrenalectomy They noted that such lesions do not in themselves result in adrenal atrophy Other workers are in good general agreement that lesions in the floor of the third ventricle (median eminence posterior tuber cinereum or mammillary bodies) may abolish the discharge of

The experimental basis for the suggestion is as follows Colfer de Groot and Harris (1950) found that the rabbit reacted as other forms in that emotional stress stimuli, such as restraint or subcutaneous faradism, evoke a lymphopenia Hypophysectomy abolished this response to stress though injection of A C T H was still followed by a lymphopenia in the hypophysectomized animal There was then available for investigation a reaction which could be indicated emotional stress → anterior pituitary gland → adrenal cortex → lymphopenia To see whether A C T H secretion could be evoked by electrical stimulation of the hypothalamus de Groot and Harris (1950) used the remote control technique with the primary coil enlarged to surround the cage containing the animal (see Chapter 4) Thus the animal was not anaesthetized and was not subjected to concurrent emotional stress during the periods of stimulation It was found that stimulation of the posterior region of the tuber cinereum or mammillary body resulted in a lymphopenia that was similar in time relation and magnitude to that following an emotional stress stimulus or intravenous injection of an appropriate dose of A C T H Stimulation of other regions in the hypothalamus (including the supraopticohypophysial tract in the anterior part of the median eminence) and pituitary gland (pars and zona tuberalis pars distalis pars intermedia and infundibular stem) did not elicit the response Results which are in the main similar to these have been obtained by Hume and Wittenstein (1950) by remote control stimulation of the hypothalamus of the unanaesthetized dog (see also Hume 1952) Using the eosinophile count as an indication of A C T H release they reported that stimulation of the anterior hypothalamus resulted in A C T H secretion in the intact or completely sympathectomized dog This latter observation eliminated the possibility that the stimulus was acting by exciting the secretion of adrenaline More recently Hume (1953) has reported that the stimulation of the posterior tuber cinereum of the hypothalamus of dogs also results in an eosinopenia Porter (1953 and 1954) working with the cat and monkey, found that stimulation of the mammillary bodies or posterior tuber cinereum (but not of the anterior hypothalamus) results in an eosinopenia There can be little doubt that the posterior hypothalamus is in some way functionally related to the secretion of A C T H

tion of a limb was found to abolish the effect of operative trauma in causing A C T H discharge, although severe scalds or burning of a denervated limb still resulted in anterior pituitary activation (Gordon 1950*b* Hume 1953) The work of Porter (1952 1954) is also compatible with the view that systemic stresses need an intact hypothalamus to mediate discharge of A C T H Working on cats and monkeys, under cyclopropane anaesthesia Porter was able to demonstrate a marked increase in the electrical activity of the posterior hypothalamus at the level of the mammillary bodies following a systemic stress stimulus (injection of adrenaline or insulin or conditions of hypoxia) The more recent observations of Bonvallet Dell and Hiebel (1954) throw doubt on the specificity of this hypothalamic response Also recent unpublished work (Fortier Harris McDonald—see Harris, 1955) has shown that pituitary stalk section in the rabbit results in marked adrenal atrophy and in the loss of the normal A C T H discharge in response to the stress of restraint or cold exposure whilst injection of adrenaline or laparotomy still results in a lymphopenia or reduction of adrenal ascorbic acid These data are in line with Fortier s original thesis However these results could also be explained on the assumption that all stress stimuli act through the nervous system and that intense stimuli are capable of causing the liberation of sufficient humoral transmitter from the median eminence into the systemic circulation to excite the isolated or stalk cut pituitary gland This view seems unlikely on the grounds that any humoral transmitter normally carried by the portal vessels would be so diluted in the systemic circulation as to be unable to produce any effect on pituitary cells The results of Lynch Keller Batsel Witt and Galvin (1952) that removal of the proximal part of the pituitary stalk and the ventral half of the hypothalamus (including the median eminence) does not prevent an eosinopenic response following surgical trauma in dogs also argues against this possibility As a working hypothesis for the present it is felt that stresses may be divided into two types Firstly neural stresses—including the majority of stimuli arising in the external environment—which act through the central nervous system and vessels of the pituitary stalk to cause pituitary discharge of A C T H and secondly systemic stresses—which include those noxious stimuli that result in tissue damage or metabolic disturbance—

A C T H following stress For example McCann (1953) and Laqueur McCann Schreiner Rosemberg, Anderson and Rioch (1953) working on the rat and cat respectively found that lesions in the basal infundibular hypothalamus *decreased or abolished* the adrenal cortical response to injection of adrenaline or opera tive trauma Lesions in the mammillary bodies were associated with normal eosinopenic responses to stress Porter (1953 and 1954) using cats and monkeys, found that lesions of the posterior tuber cinereum and mammillary bodies prevented the eosinopenic response to injections of adrenaline formalin and histamine in cats, and to adrenaline histamine and surgical trauma in mon keys This localization of the active area is in good agreement with the findings derived from electrical stimulation of these regions

From the results of experiments involving electrical stimula tion or lesions it seems probable that the hypothalamus is in volved in the transmission of the stimulus of stress to the anterior pituitary gland It is not yet clear though whether all types of stress act in this or only in this manner Fortier (1951) on the results of his experiments on pituitary transplants divided stresses into two groups (*a*) systemic (injection of adrenaline or histamine exposure to cold) and (*b*) neurotropic (exposure to loud sounds immobilization) According to this worker sys temic stresses elicit A C T H discharge by an action on the anterior pituitary via the general blood stream and neurotropic *stresses require the mediation of the central nervous system in* order to effect A C T H release This view is partly opposed and partly supported by more recent findings Several investi gators have reported that hypothalamic lesions may block the release of A C T H to Fortier s systemic type of stress For example, the release of A C T H which follows injection of adrenaline was found absent after the placement of various hypo thalamic lesions (Hume 1952 McCann 1953 Porter 1953 and 1954 Laqueur et al 1953) Also Briggs and Munson (1954) have reported *that the discharge of A C T H that follows injection* of adrenaline or surgical trauma is very largely if not completely abolished by pretreatment of rats with morphine Morphine was shown not to interfere with the normal response to exogenous A C T H and it seems probable that it exerts the above action by inhibiting some reflex nervous excitation Further denerva

sinusoid like connections become re established across a cleanly divided pituitary stalk with rapidity (within a few days in the case of the rat) and in a high proportion of cases In view of these findings the significance of many early experiments dealing with stalk section is doubtful Bearing these points in mind a search of the literature has failed to reveal any reliable data indicating normal secretion of A C T H (or other anterior pituitary hormone) under basal or stress conditions in the absence of hypophysial portal vessels connecting the median eminence and anterior pituitary gland Similar observations may be made in regard to the adrenocorticotrophic function of anterior pituitary transplants and grafts in hypophysectomized animals Adrenal glands of normal size weight and histology have only been observed in such animals when the graft is placed under the median eminence or cut pituitary stalk and when it is vascularized by regenerated portal vessels

(D) Participation of the cerebral cortex in regulating A C T H secretion

It has seemed probable for some time that the cerebral cortex is in some way concerned with the rate of release of A C T H since states of emotional stress such as anxiety worry or excitement are potent stimuli in increasing the secretion of this hormone Only recently has direct evidence been forthcoming regarding this relationship of the cerebral cortex Porter (1954) has reported the following observations made on monkeys anaesthetized with cyclopropane

1 Stress stimuli which increased the electrical activity of the tuberal and mamillary regions of the hypothalamus activated also the anterior nucleus of the thalamus and the anterior cingulate gyrus The increased activity of these regions was dependent on the presence of an intact hypothalamus The probable correlation was drawn between these findings and the well established anatomical paths linking the mammillary bodies anterior nuclei of the thalamus and the anterior cingulate gyrus

2 Electrical stimulation of the orbital surface of the frontal lobe resulted in a marked eosinipenia

3 Electrical stimulation in the hippocampal region in particular the uncus inhibited the eosinopenia which normally followed administration of adrenaline or operative trauma

that normally act *both* through the nervous system and by producing changes in blood chemistry

The pathway by which a stimulus passes from the hypothalamus to the anterior pituitary appears to be by means of the hypophysial portal vessels The work quoted above on the adrenocorticotrophic activity of different types of anterior pituitary transplants and grafts renders this view most probable as does the work of de Groot (1952) who studied the effect of different types of stress stimuli at varying periods after cutting the pituitary stalk in mice de Groot found that mice respond to a stress stimulus with a lymphopenia most marked at the third hour after the stimulus Following simple section of the pituitary stalk this lymphopenic response was temporarily abolished but slowly re established between the 6th and 15th day after operation Histological study of the pituitary stalk of these animals made after vascular perfusion with indian ink and serial sectioning of the hypothalamic pituitary region revealed that the hypophysial portal vessels regenerate across the site of the stalk section in a manner similar to that seen in rats and monkeys and that the magnitude of the lymphopenic response to stress could be correlated with the degree of vascular regeneration In a further series of animals section of the pituitary stalk with the placement of a waxed paper plate between the cut ends was *found to abolish the lymphopenic response to stress for the duration* of the experiment (11 days) Marked atrophy of the adrenal glands was also noted provided the plate was in a position to prevent portal vessel regeneration These experiments indicate *that the increased release of* A C T H *in response to stress* stimuli requires an intact vascular connection between the median eminence and the anterior pituitary

The view that the hypothalamus controls release of A C T H by means of the hypophysial portal vessels has been criticized on the grounds that section of the pituitary stalk or hypophysectomy with transplantation of the anterior pituitary gland to a site remote from the sella turcica does not abolish all signs of A C T H secretion In considering results based on experiments involving section of the pituitary stalk attention should be paid to the possibility of regeneration of the portal vessels Such regeneration has now been observed in the mouse rat rabbit ferret and monkey and it may be said that capillary and large

(1) *Under optimum quiescent conditions* the main stimulus to A C T H secretion appears to be some hypothalamic drive mediated via the hypophysial portal vessels (data derived from hypophysial transplants and grafts) The systemic blood concentration of adrenal cortical hormone probably helps maintain a more constant basal secretion of A C T H

(2) *Under conditions of stress* it seems likely that one or both of two mechanisms may be brought into play Firstly, what may be called neural stress stimuli appear to excite A C T H liberation by means of nervous reflex paths acting through the hypothalamus and hypophysial portal vessels And secondly systemic stresses that cause tissue damage or metabolic changes probably excite A C T H discharge both by neural reflex activation of the hypothalamus and by producing changes in the chemistry of the systemic blood which in turn affects pituitary tissue directly It is probable that release of adrenaline may play a subsidiary role in exciting A C T H release There are few data concerning the peripheral utilization of adrenal cortical hormone what data are available indicate that variations in blood level of adrenal cortical hormone do not play a part in the stress induced secretion of A C T H

Fortier (personal communication) suggests that the function of the pituitary stalk so far as the pituitary adrenal axis is concerned is to form the connecting link between the external environment and central nervous system on the one hand and the anterior pituitary gland on the other The adrenal cortical atrophy that follows pituitary stalk section he suggests may be explained as due to the removal of tonic environmental stimuli which normally act to maintain A C T H secretion

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These findings await confirmation Their possible significance for psychiatry, and for neuroendocrine relations in general, is obvious and it is to be hoped that further information may be obtained in the near future on unanaesthetized animals

(E) Conclusions regarding the control of A C T H secretion

The most likely view, at the present time concerning the factors regulating A C T H secretion are represented diagrammatically in Fig 42

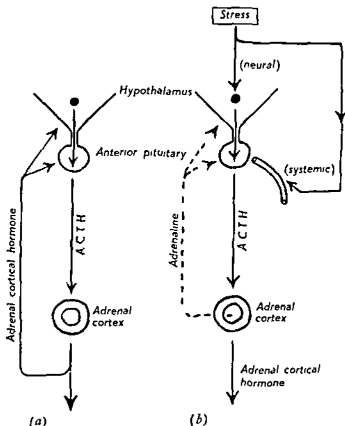


Fig 42 (*a*) Factors regulating the basal quiescent secretion of A C T H (*b*) Factors responsible for the increased discharge of A C T H under stressful conditions Under these circumstances two components appear to play a part in pituitary excitation a neural component acting through the pituitary stalk and a systemic component acting through the systemic blood supply of the gland

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CHAPTER 6

REGULATION OF THE SECRETION OF THE THYROTROPHIC, LACTOGENIC AND GROWTH HORMONES

Thyrotrophic Hormone (T S H)

(A) Thyroid activity and the central nervous system

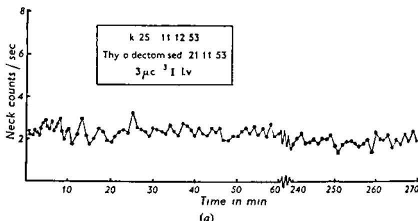
(i) *Clinical data* Ever since the first description of Graves disease by Parry (1825) and Graves (1835) psychic trauma has received prominence as an important factor preceding the onset of this condition The correlation between emotional shock and the onset of hyperthyroidism has been described many times Lidz and Whitehorn (1950) have recently attempted to analyse the type of emotional disturbance involved and conclude that Graves disease frequently occurs in individuals that are highly dependent on interpersonal relationships for their security and that disruption or the threat of disruption of such relationship is apt to precipitate the disease In spite of so many accounts emphasizing the importance of emotional stress it is perhaps wise not to accept the fact as firmly established since comparable control series of cases have rarely been published However the view *is often expressed that the overactivity of the thyroid is* secondary to anterior pituitary stimulation which is in turn probably related to some disturbance in the hypothalamus (Thompson 1948) The view is implicit in the writings of many clinicians that an emotional shock has resulted in nervous reflex stimulation of pituitary thyrotrophic hormone and that this is at least a precipitating cause of the hyperthyroidism

(ii) *Experimental data* Experimental work has demonstrated clearly an effect of central nervous activity and stress on the thyroid gland *The well known adjustments of thyroid activity* to changes in the environmental temperature are in all probability mediated at least in part through the nervous system The effect of increased or decreased exposure to light in varying the activity of the thyroid (Puntriano and Meites 1951) and the evidence that coitus stimulates the release of thyrotrophin in the

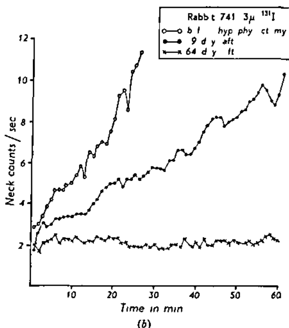
rabbit (Saxton and Greene 1942) might be taken to indicate a neural influence over thyroid activity

In most recent work radioactive iodine has been used to measure the activity of the thyroid gland Following an injection of ^{131}I the iodine diffuses into the tissue spaces of the body is taken up by the thyroid gland and is excreted by the kidney Forty eight hours after an injection of a tracer dose of ^{131}I (into a rabbit) the tissue fluid blood and urine content have fallen to negligible values and the only organ in the body containing appreciable amounts is the thyroid gland which contains ^{131}I in organic combination A few hours after the initial injection the thyroid starts secreting radioactive hormone into the blood This hormone is in the form of an organic compound and is often referred to as the blood protein bound iodine or P B I Two simple methods are available for assessing thyroid activity using ^{131}I Firstly the rate at which the thyroid takes up radio iodine from the blood may be measured and secondly the rate at which radioactive hormone is lost from the gland may be studied Such techniques are applicable to intact conscious animals and examples will be briefly mentioned

The rate of uptake of ^{131}I The rate at which the thyroid gland extracts radio iodine from the blood will depend not only on thyroid activity but also on the tissue fluid space the rate of renal excretion of iodine and on the blood level of ^{127}I This latter will in turn depend on the general iodine level of the diet It is therefore necessary to perform measurements on thyroid ^{131}I uptake on animals under standardized conditions of environmental temperature and lighting and on a constant diet Further if the thyroid content of ^{131}I is taken to reflect the total amount of ^{131}I removed from the blood it is necessary to complete the measurements within 1–2 hours since after this time ^{131}I will begin to be lost from the gland as radioactive hormone Brown Grant and Gibson (unpublished observations) have recently used a scintillation counter and a rate meter for obtaining repeated measurements of the radioactive iodine content of the thyroid glands of rabbits following injection of a tracer dose of ^{131}I The animals were immobilized in a hammock and the counter arranged in a constant geometric relationship to the gland In this way curves as illustrated in Fig 43 have been obtained It may be seen (Fig 43*a*) that injection of ^{131}I into a



(a)



(b)

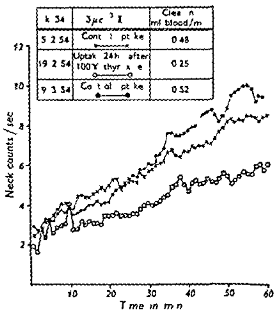
Fig 43 Curves representing the uptake of ^{131}I by the thyroid gland of the rabbit following injection of a tracer dose of ^{131}I (3 μc 1 v)

(a) Curve of radioactivity of the thyro d region of a thyroidectomized rabbit

(b) (c) (d) illustrate the effects of hypophysectomy injection of thyroxine and injection of thyrotrophic hormone on the thyroid uptake of ^{131}I

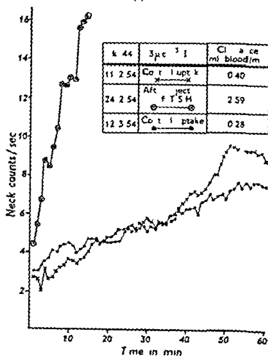
Serial estimations of blood radioactivity during the course of these experi ments enable figures to be given for the thyroid clearance of blood radio iodine (c and d)

(From Brown Grant and Gibson unpublished)



k 54
$3\mu c$ ^{3} I
Clea n ml blood/m
5 2 54
Cont l pt ke
0 48
19 2 54
Uptak 24h after 100Y thyr x e
0 25
9 3 54
Co t al pt ke
0 52
Neck counts / sec
T me in m n

(c)



k 44
$3\mu c$ ^{3} I
Cl a ce ml blood/m
11 2 54
Co t l upt k
0 40
24 2 54
Aft ject f T S H
2 59
12 3 54
Co t l ptake
0 28
Neck counts / sec
T me in min

(d)

thyroidectomized rabbit results in the recording of a steady level of radioactivity from the thyroid region Reference to the con trol experiments depicted in Figs 43*b* *c* *d*, shows that in the normal rabbit a steady increase of radioactivity in the thyroid region follows injection of radio iodine The rate of this ^{131}I uptake is decreased by hypophysectomy (Fig 43*b*) or previous administration of thyroxine (Fig 43*c*) and increased by injection of thyrotrophic hormone (Fig 43*d*) In order to make this method independent of the exact dose of ^{131}I given or of changes in renal excretion of ^{131}I serial blood samples were taken and the radioactivity of these samples measured These data enable figures to be obtained for the thyroid clearance of blood radio iodine as indicated in Figs 43*c* and *d*

The rate of release of radioactive hormone In 1951 three groups of workers (Wolff 1951 Albert 1951 Perry 1951*a*) published a method of measuring thyroid activity by studying the rate of loss of organically bound ^{131}I from the thyroid gland of rats In a study of the factors which modify thyroid activity Brown Grant, von Euler Harris and Reichlin (1954) have made use of measurements of the output of ^{131}I from the thyroid gland of the rabbit The thyroid radio iodine was measured twice daily by placing the animal (unanaesthetized) in a hammock with the thyroid region of the neck in a constant relationship to a Geiger Muller tube On plotting the log of the neck counts so obtained against time a straight line relationship was found (Fig 44*a*) The slope of this line is proportional to the

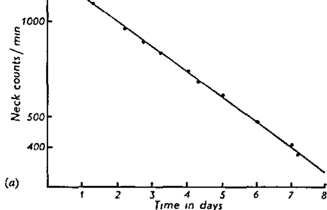
$$\frac{\textit{amount of hormone secreted per unit time}}{\textit{amount of hormone in the gland}}$$

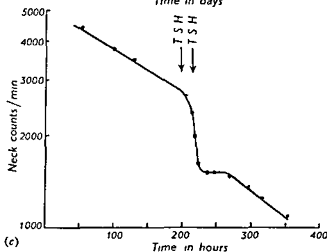
Providing therefore that the total amount of hormone in the gland remains constant the slope of the curve may be taken as related to the amount of hormone secreted per unit time that is to thyroid activity One minor error exists in the fact that radio active hormone is partly degraded with liberation of ^{131}I and that a proportion of this radio iodine is re accumulated by the thyroid However a detailed study showed that under normal circumstances only about 10 per cent of the radioactivity lost from the thyroid in any one day is re accumulated as ^{131}I Brown Grant et al submitted rabbits to a variety of procedures known to affect thyroid activity and observed the effects on such

output curves It was found that cold usually (but not always) accelerated the rate of loss of radio iodine injection of T S H accelerated and injection of thyroxine or hypophysectomy inhibited such loss (see Figs 44*b c d e*) Fig 44*f* shows the effect of administration of thyrotrophic hormone on the very slow rate of release of thyroidal ^{131}I of the hypophysectomized animal Methods based on the measurement of thyroid output of ^{131}I have several advantages over methods based on thyroid uptake of ^{131}I in that they give a measure of slow changes in thyroid activity that they are less dependent on changes in the level of ^{131}I in the blood or on changes in renal excretion of iodine and that each experiment is of sufficient duration to allow control and experimental periods of observation

The effect of noxious or stressful stimuli on thyroid activity has been the subject of many studies Most workers have found that varied types of stress will diminish the uptake of radioactive iodine by the thyroid glands of rats (Williams Jaffe and Kemp 1949 Paschkis Cantarow Eberhard and Boyle 1950 Bogoroch and Timiras 1951 Middlesworth and Berry 1951 and others) The stress stimulus used in these experiments was of varied types (injection of adrenaline or typhoid vaccine trauma injection of formalin anoxia starvation cervical cordotomy forced muscular exercise) but all are reported to inhibit the uptake of ^{131}I by the thyroid gland However observations made only on the uptake of thyroidal ^{131}I may be misleading in this respect since it is known that stress leads to adrenal cortical activation and that stress or administration of cortisone (Bogoroch and Timiras 1951 Ingbar 1953) increase the rate of renal excretion of radio iodine Such changes in renal excretion might lead to a decreased thyroid uptake of ^{131}I that does not reflect diminished thyroid activity However Gibson Harris and Skynner (unpublished) have found that prolonged (36–44 hr) emotional stress in rabbits produced by restraint results in a decreased ^{131}I uptake and also decreased clearance of blood ^{131}I by the thyroid (Fig 45*a*)

A detailed study of the effect of emotional and physical stresses on thyroid activity of the rabbit have been made by Brown Grant Harris and Reichlin (1954*a*) using measurements of the rate of release of thyroidal ^{131}I In these experiments it was found that rabbits subjected to emotional stress (48-hr





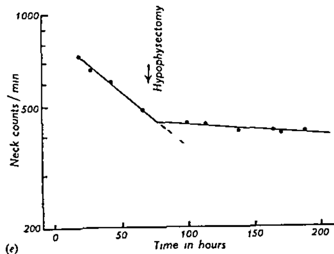


Fig 44 (a) (b) (c) (e) and (f) from Brown Grant von Euler Harris and Reichlin (1954) *J Physiol* 126 1)

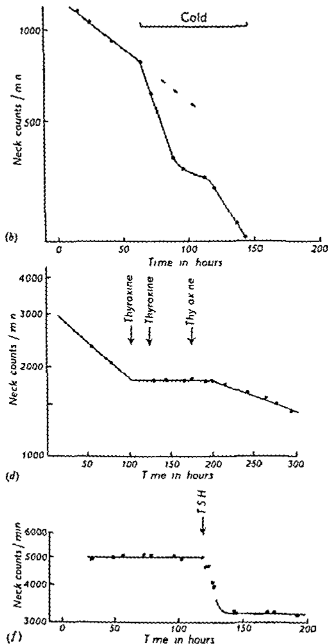


Fig 44 (a) Rabbit 343 21 4 53 Normal release curve Rabbit injected with 2 μc ^{131}I and 48 hours later (that is at zero time on the abscissa) neck counts are started Note that over the ensuing eight days the radioactivity of the thyroid decreases exponentially In this and other figures of release curves the neck counts obtained have been corrected for physical decay of ^{131}I

(b) Rabbit 367 18 11 52 (c) Rabbit 344 23 4 52 (d) Rabbit 344 2 5 52 and (e) Rabbit 542 14 5 53 show respectively the effect of cold exposure injection of thyrotrophic hormone and thyroxine and hypophysectomy on the release curve

(f) Rabbit 54 hypophysectomized one month previously shows the effect of injection of thyrotrophic hormone on the release curve of the hypophysectomized rabbit

periods of restraint produced by tying the animal s back legs to gether or to the side of the cage placing the animal in a small cage that is then replaced in the animal s usual cage varying the conditions of environmental lighting or by administering infre quent subcutaneous faradic shocks) or to physical or systemic stress (injection of irritating solution into the peritoneal cavity, haemorrhage or laparotomy) consistently resulted in thyroid in hibition (Fig 45 *b* and *c*) These changes in thyroid secretory rate were similar to those obtained after injection of thyroxine in that they occurred after a short latent period of, at most a few hours It was noticed that prolonged application of a constant emotional stress stimulus often resulted in thyroid 'escape from the inhibition and that adaptation might also follow if some emotional stimuli were repeatedly applied in subsequent experiments These results, and those quoted above demon strate that the thyroid gland reacts quickly and in a labile manner to external stimuli The fact that it may react rapidly to mild emotional stimuli suggests that in some way the activity of the thyroid is influenced by the central nervous system

In view of the above results it is difficult to explain the findings of Kracht and Kracht (1952) and Kracht (1954) who described a condition of fright thyrotoxicosis occurring in wild rabbits brought into captivity These findings have not been confirmed however Brown Grant Harris and Reichlin (1954*a*) reported that wild rabbits caught in nets and brought into captivity re mained healthy and did not develop any signs of hyperthyroid ism On subjecting these animals to restraint an inhibition of ^{131}I release was observed in these as in tame, rabbits

(B) Pathway of central nervous influence over thyroid activity

There are at least three possible pathways by which emotional stress and central nervous activity may affect the thyroid gland

(1) *Cervical sympathetic system* The thyroid gland receives nerve fibres from the cervical sympathetic system and the vagi (via the laryngeal nerves) (Nonidez 1931 Ross and Moorhouse 1938) The site of ending of these fibres is doubtful In spite of *descriptions* that individual follicular cells of the gland are sur rounded by nerve nets (Sunder Plassman 1934) the overall evidence seems to be against the presence of such structures

(Nonidez 1935) Holmgren and Naumann (1949) studied the nerves in the thyroid glands of guinea pigs after staining with methylene blue or silver methods They describe perivascular plexuses interfollicular plexuses and myelinated fibres running through the parenchyma mostly of sympathetic origin No direct contact between nerve fibres and epithelial cells was observed Vagotomy or sympathectomy did not affect the height of the follicular cells the histology of the gland or the response to thyrotrophic hormone There are many papers in the literature of thirty to forty years ago dealing with the effects of section of or stimulation of the cervical sympathetic system on thyroid activity Changes in thyroid histology in the output of hormone as measured in thyroid venous blood and in metabolic rate were studied following these procedures Looking back at this work from the vantage point of later years the results obtained appear to be of doubtful significance Much discussion centred at one time around the results reported by Cannon Binger and Fitz (1915) and Cannon and Fitz (1916) These workers described a state in cats resembling Graves disease in man that occurred following anastomosis of the central end of the cut phrenic nerve with the distal end of the cervical sympathetic trunk Later workers were unable to repeat these results and in further studies Friedgood and Cannon (1940) obtained a similar effect in only 2 out of 28 cats In this account Friedgood and Cannon remark that the balance of evidence is considerably against any direct secretory nerves to the thyroid and that the changes observed may have been due to vasomotor changes or possibly alterations in pituitary activity Similar views are expressed on the whole by Friedgood and Bevin (1939) Uotila (1939*a*) Brock Doty Krasno and Ivy (1940) and Lowe Ivy and Brock (1945) who investigated the relationship between the cervical sym pathetic system and the thyroid gland

The usual inhibition of thyroid activity produced by stress stimuli occurs in rabbits that have been subjected to bilateral stellate ganglionectomy (Brown Grant et al 1954*a*) (Fig 45*c*) This finding indicates that the inhibitory effect of stress is not mediated by a sympathetic nerve supply to the thyroid gland

Data derived from studies of thyroid transplants also demon strate the fact that a nerve supply is not essential for the normal function of this gland

periods of restraint produced by tying the animal s back legs together or to the side of the cage placing the animal in a small cage that is then replaced in the animal s usual cage varying the conditions of environmental lighting or by administering infrequent subcutaneous faradic shocks) or to physical or systemic stress (injection of irritating solution into the peritoneal cavity, haemorrhage or laparotomy) consistently resulted in thyroid inhibition (Fig 45 *b* and *c*) These changes in thyroid secretory rate were similar to those obtained after injection of thyroxine in that they occurred after a short latent period of, at most a few hours It was noticed that prolonged application of a constant emotional stress stimulus often resulted in thyroid escape from the inhibition and that adaptation might also follow if some emotional stimuli were repeatedly applied in subsequent experiments These results and those quoted above demonstrate that the thyroid gland reacts quickly and in a labile manner to external stimuli The fact that it may react rapidly to mild emotional stimuli suggests that in some way the activity of the thyroid is influenced by the central nervous system

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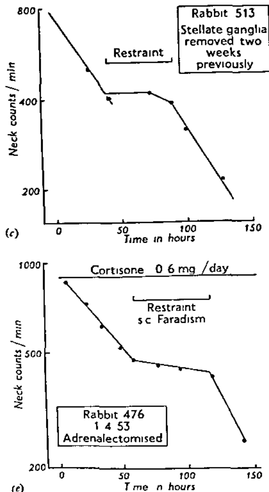


Fig 45 (*a*) To show th effe t of p ev ous mot onal stress (39 hour restra nt) on the rate of upt ke of ^{131}I in the rabb t The insert figures gi e the average blood clearanc of ^{13} I by the thyro d during the th e experiments

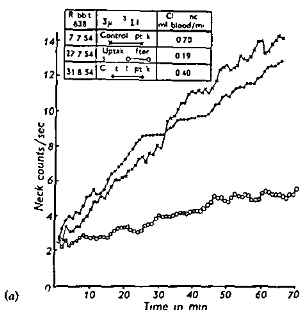
(*b*) Rele se curve to show the effect of emot onal stress (restraint and sub cutaneous f radism) upon the thy o dal release of ^{131}I in the normal rabbit

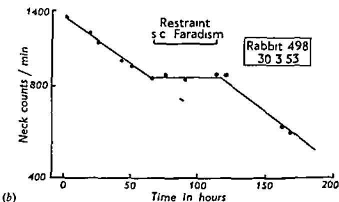
(*c*) Release curve to show tl e effect of estra nt upon the thyro dal release of ^{3} I n the rabbit ft r b l ter l removal of the stellate gangl a

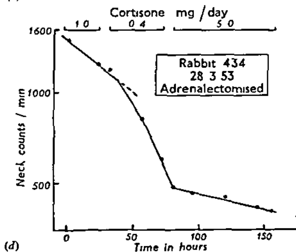
(*d*) Rele e curve of an adrenalectom ed r bb t to show the effect of varying the m nten nc dose of cort sone

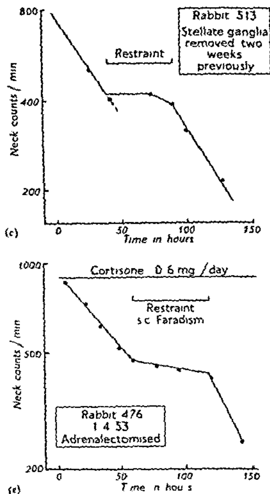
(*e*) R lease curve to llustrate the effect of emot onal stress on the thyro dal release of ^{131}I of the drenalectom zed bbit on a constant cortisone regime

(Fig 45 (*a*) from Gibson Harr s & Skynne 1954 unpublish d (*b*)–(*e*) from B o n G ant Harr s & Re chl n (1954 *a* and *b*) *J Phy ol* 126 29 and 41)


Neck counts/sec
Time in min
(a)


Restraint
s c Faradism
Rabbit 498
30 3 53
Neck counts / min
Time in hours
(b)


Cortisone mg / day
1 0
0 4
5 0
Rabbit 434
28 3 53
Adrenalectomised
Neck counts / min
Time in hours
(d)



F g 45 (*a*) To show the effect of prev ous emotional stress (39 hour restraint) on the rate of uptake of ¹³¹I in the rabb t The insert figures g ve the average blood clearance of ¹ I by the thyro d during the three experiments

(*b*) Release curve to show the effect of emotional stress (restraint and sub cutaneous faradism) upon the thyroidal release of ¹³¹I n the *normal* rabbit

( ) *Release curve to show the effect of restra nt upon the thyroidal release of* ¹³¹I in the rabbit after b lateral removal of the stellate ganglia

(*d*) Release curve of an ad enalectomized rabbit to show the effect of varying the maintenance dose of cort sone

(*e*) Release curve to llust ate the effect of emotional stress on the thyroidal release of ³¹I of the ad enalectomized rabbit on a constant cortisone regime

(Fig 45 (a) from G bson Ha ris & Skynne 1954 unpublish d (*b*)–(*e*) from B own Grant Harris & Re chlin (1954 *a* and *b*) *J Phy ol* 126 29 and 41)

(ii) *C N S* → *Adrenal gland* → *Thyroid* Since conditions of stress are known to excite secretory activity of the adrenal medulla and cortex it is possible that the thyroid inhibition occurring under the same circumstances is secondary to and dependent on an increased blood level of adrenaline or adrenal cortical hormone There is certainly evidence that injection of adrenaline A C T H or cortisone will reduce the uptake of ^{131}I by the thyroid glands of rats or man (Williams Jaffe and Kemp 1949, Soffer Gabrilove and Jailer 1949 Money Kirschner Kraintz Merrill and Rawson 1950 Reiss Riggs Thorn and Forsham 1950 Money Kraintz Fager Kirschner and Rawson 1951 Berson and Yalow 1952 and others) So marked is this effect that administration of A C T H and cortisone has been tried as a therapeutic measure in cases of Graves disease and good results have been reported (Hill Reiss Forsham and Thorn 1950 Reiss Riggs Thorn and Forsham 1950)

The effect of adrenaline A C T H and cortisone on the rate of release of radio iodine from the thyroid gland would also indicate that these hormones exert an inhibitory effect on thyroid activity The earlier work (Perry 1951*b* Albert Tenney and Ford 1952) indicated that A C T H and cortisone do not depress the release of radio iodine from the glands of rats Recent work on the rabbit however (Brown Grant Harris and Reichlin 1954*b*) has demonstrated that the administration of large doses of adrenaline or small doses of A C T H or cortisone (but not desoxycorticosterone acetate) can markedly inhibit the release of thyroid radio iodine in both the normal or bilaterally adrenalectomized animal (Fig 45*d*) The most accurate results in experiments of this type are probably obtained from experiments on adrenalectomized animals in which the total quantity of circulating adrenal steroid can be more accurately controlled Similar results concerning the action of cortisone on thyroid activity (^{131}I release) of rabbits have been obtained by Myant (1953) Further Brown Grant (unpublished) has found in contradiction to the results of Perry and Albert et al mentioned above an inhibitory action of cortisone on the release of radio iodine from the thyroid of rats

It seems clear however that the thyroid inhibitory action of adrenaline or adrenal cortical steroids does not play an essential part in the response of the thyroid gland to stress stimuli Bı

laterally adrenalectomized rabbits maintained on constant corti sone dosage still respond to stress with a decreased release of thyroid hormone (Fig 45*e*) (Brown Grant Harris and Reichlin 1954*a*) This observation does not eliminate the possibility that stress stimuli induce alteration in the rate of tissue utilization of adrenal corticoids and thereby effect thyroid activity However this appears unlikely since such an explanation would imply a *reduction* in tissue utilization of corticoids under conditions of stress It is impossible to say whether the increased blood level of adrenal cortical hormone which follows the application of stress to the normal animal plays a subsidiary or supporting role in producing the thyroid response the fact that changes in thy roid activity may be produced by administration of amounts of cortisone that appear to be within the physiological range would support this view

(iii) *C N S* → *T S H* → *Thyroid* The conclusion that at least some forms of stress especially emotional stress result in decreased thyroid activity by diminishing the anterior pituitary release of T S H appears inescapable The fact that the central nervous system and in particular the hypothalamus exerts a controlling influence over the secretion of gonadotrophic hor mone and A C T H makes it easier to visualize a similar control for T S H secretion This view is also compatible with the data regarding the lability of the thyroid responses to a large number of environmental stimuli (discussed above) and the speed with which changes in thyroid activity can occur in response to such stimuli

(C) The reciprocal relationship between secretion of T S H and A C T H

In general it seems that stress stimuli excite the adrenal cortex and inhibit the thyroid gland and that this reflects a reciprocal relationship between the secretion of A C T H and T S H There are two circumstances however under which the rate of secretion of A C T H and T S H *appear* to change in the same direction

(i) *Cold* It is well known that exposure to a cold environment increases the rate of secretion of A C T H and also increases thyroid activity It is possible then that cold forms an exception amongst stress stimuli in so far as it causes an increased rate of

(ii) *C N S* → *Adrenal gland* → *Thyroid* Since conditions of stress are known to excite secretory activity of the adrenal medulla and cortex it is possible that the thyroid inhibition occurring under the same circumstances is secondary to and dependent on an increased blood level of adrenaline or adrenal cortical hormone There is certainly evidence that injection of adrenaline A C T H or cortisone will reduce the uptake of ^{131}I by the thyroid glands of rats or man (Williams Jaffe and Kemp 1949 Soffer Gabrilove and Jailer 1949 Money Kirschner Kraintz Merrill and Rawson 1950, Reiss Riggs Thorn and Forsham 1950 Money Kraintz Fager Kirschner and Rawson 1951 Berson and Yalow 1952 and others) So marked is this effect that administration of A C T H and cortisone has been tried as a therapeutic measure in cases of Graves disease and good results have been reported (Hill Reiss, Forsham and Thorn 1950 Reiss Riggs Thorn and Forsham 1950)

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It seems clear however that the thyroid inhibitory action of adrenaline or adrenal cortical steroids does not play an essential part in the response of the thyroid gland to stress stimuli Bi

treatment results in decreased secretion of A C T H The effect of thiouracil in producing adrenal cortical atrophy would seem to be particularly significant since administration of foreign sub stances so commonly results in increased A C T H discharge

The above findings recall to mind the older views of Marine and others that the hyperactive thyroid of the Graves patient is to be correlated with atrophy of the adrenal cortex In this respect the following observations appear to be relevant

(i) Adrenal insufficiency in rabbits (by removal of or freez ing the adrenal glands) results in increased heat produc tion and respiratory exchange and a symptom complex resembling exophthalamic goitre (Marine and Baumann 1921)

(ii) Thyrotoxicosis is often associated with signs of adreno cortical underactivity (large thymus lymphoid hyper-plasia) and a small adrenal cortex (Marine 1930)

(iii) Graves disease has been noted to follow X ray damage to the adrenal cortex (Oppenheimer 1937) and the incidence of this condition has been reported as ten times greater in patients suffering from Addison s disease than in normals (Frederickson 1951)

(iv) Patients suffering from Cushing s syndrome may show signs of hypothyroidism (high level of serum cholesterol dry skin and low basal metabolic rate) (Heinbecker 1944) and an inactive thyroid (Cushing 1933)

It would seem that there are good reasons for believing that the adrenal cortex is in some way involved in Graves disease Whether the atrophy of the adrenal cortex is involved from the aetiological point of view or whether it is a consequence and part of the developed condition remains to be seen

An interesting speculation is that the patient with Graves disease has responded to stress with the opposite response of the normal That is that some stress stimulus has resulted in in creased thyroid and decreased adrenal cortical activity Such a view would suggest that some neurological mechanism *is prim arily* at fault and would indicate that the condition is really a disease rather than the result of an exaggerated physiological response

secretion of both hormones However this conclusion does not necessarily follow since there are few data on record regarding simultaneous measurement of adrenal cortical and thyroid activity on exposure to different grades of cold In the work of Brown Grant von Euler Harris and Reichlin (1954) rabbits were exposed to varying degrees of cold and it was found that 10 of 12 rabbits placed from 29° C to 15–21 C showed an increased *thyroidal* ^{131}I *output as compared* with 2 out of 7 rabbits exposed to a temperature of 1° C The only 3 rabbits in the series to show inhibition of thyroid activity following cold were those placed in a room at 1 C and subjected to a continuous draught of air Now exposure to a temperature of about 0 C is probably a more effective stimulus to A C T H release than exposure to a temperature of 15–21 C It is thus possible as suggested by Bogoroch and Timiras (1951) that minor degrees of cold exert a specific effect on T S H release and increase thyroid activity whereas greater degrees of cold act as a non specific stress inhibiting the thyroid and activating the adrenal cortex The effect of cold (5 C) in inhibiting the thyroid gland has also been observed by Williams Jaffe and Kemp (1949) Further and more detailed work on this subject is required

(ii) *Injection of cortisone* It is well established that injection of cortisone inhibits or tends to inhibit the pituitary secretion of A C T H Brown Grant Harris and Reichlin (1954*b*) found that administration of cortisone to rabbits markedly inhibits the rate of release of ^{131}I from the thyroid and produced evidence that this effect was due to inhibition of T S H secretion Injection of cortisone then appears to depress both A C T H and T S H release

Thyrotrophic secretion would appear to be inhibited under circumstances leading to increased secretion of A C T H It may also be true that when T S H secretion is increased A C T H liberation is decreased As an example of this latter relationship the results seen to follow administration of thiourea or thiouracil may be quoted Administration of antithyroid compounds results in increased discharge of pituitary thyrotrophic hormone and adrenal cortical atrophy (Leblond and Hoff 1944 Baumann and Marine 1945 Zarrow and Zarrow 1951) Since the atrophic adrenal gland of the thiouracil treated rat retains its sensitivity to A C T H Zarrow and Zarrow (1951) concluded that thiouracil

may secrete two thyrotrophic hormones one a growth factor dependent on the hypothalamus for its secretion and the other a metabolic factor which is independent of hypothalamic control In a more recent publication Greer Scow and Grobstein (1953) describe experiments in which anterior pituitary tissue was transplanted to the eye of hypophysectomized mice They found that the body weight and the weights of the thyroid ovaries adrenals and uterus did not differ in these animals from those of hypophysectomized controls On the other hand the pituitary transplants were found to maintain a radio iodine uptake per unit thyroid weight and a thyroid/serum iodide ratio at 2/3 the level of the intact controls Again there appeared to be a difference between the growth factor and metabolic factor in thyrotrophic activity It would seem advisable however to confirm the activity of these atrophic thyroids by other techniques before assuming that the pituitary maintains an active thyroid iodine metabolism in the absence of hypothalamic connections According to Ganong Frederickson and Hume (1954) lesions placed in or just above the anterior end of the median eminence of dogs depress the thyroid uptake of ^{131}I to within the range seen in hypophysectomized animals The atrophy of the thyroid found by Greer Scow and Grobstein to follow transplantation of anterior pituitary tissue to the anterior chamber of the eye of mice confirms the results found by Harris and Jacobsohn (1952) in which pituitary tissue was transplanted under the temporal lobe of the brain of hypophysectomized rats As described previously (Chapter 3) transplants placed outside the pituitary capsule but under the median eminence of the hypothalamus where they became vascularized by the hypophysial portal vessels supported a normal thyroid histology It is of interest that Bogdanove and Halmi (1953) and Ganong Frederickson and Hume (1954) report that hypothalamic lesions may according to their site affect thyrotrophic and gonadotrophic secretion independently

Section of the pituitary stalk has been found to have variable effects on thyroid activity in the hands of different workers The same criticisms apply to much of this work as given in Chapter 4 in respect to stalk section and reproductive activity As examples of such divergent results it may be mentioned that Uotila in much quoted work found that the thyroid gland of stalk sec-

(D) Hypothalamus and thyrotrophic secretion

Many workers have studied the effects of hypothalamic lesions pituitary stalk section and electrical stimulation of the hypo thalamus on thyroid function (see Harris 1948) Since the intro duction of more accurate methods for assessing thyroid activity much of the older work needs to be reviewed and repeated

Hypothalamic lesions have been stated to affect metabolic rate (Grafe and Grunthal 1929 Bloch 1943) Cahane and Cahane (1938) found that various infundibular lesions may produce a histological picture of increased or decreased activity in the thy roid gland From this and other evidence they postulated the presence of two centres in the infundibular region one situated between the optic chiasma and pituitary stalk which excites the secretion of the thyrotrophic hormone and the other in the tubero mammillary region which inhibits the secretion of this hormone More recent work (Greer 1952 Bogdanove and Halmi 1953) has demonstrated that destruction of a region be tween the ventromedian hypothalamic nucleus and the supra chiasmatic nucleus of the rat prevents the hypertrophy of the thyroid which normally follows thiouracil treatment This observation is of interest for two reasons Firstly it demonstrates that the hypothalamus exerts some control over T S H secre tion and secondly it indicates that variations in the blood level of thyroxine affect the rate of T S H release by an action on the hypothalamus and not directly on anterior pituitary cells In this latter connection it may be significant that radioactive thyroxine becomes concentrated in the median eminence of the tuber cinereum and the neurohypophysis in much greater amounts than in other regions (Courrier Horeau Marois and Morel 1949 Harper and Mattis 1950 1951 Jensen and Clark 1951) The further observation of Greer (1952) that the hypo thalamic lesions which are effective in preventing the hyper trophy of the thyroid following thiouracil do not affect the marked increase in thyroid serum iodide ratio which normally follows thiouracil is difficult to understand especially in view of the absence of the usual histological changes associated with the rise in thyroid activity The explanation suggested by Greer to account for this dichotomy between size and iodide concentrat ing ability of the thyroid in these animals is that the pituitary

sellar parts of the internal carotid arteries are relatively closer together than in many laboratory animals so that it was felt necessary to take further precautions to prevent regeneration of the portal vessels Therefore in some animals the right internal carotid artery was tied and cut just above the diaphragma sellae the right oculomotor nerve removed and a large waxed paper plate fixed firmly in position above the pituitary Although these studies are not yet complete results so far obtained indicate that effective pituitary stalk section results in

(*a*) Marked decrease in thyroid activity as measured by the rough test of 48 hr uptake of ^{131}I or by the slope of the release curve The thyroid activity of the stalk cut rabbit seems to remain at a slightly higher level than that of the hypophysectomized rabbit

(*b*) Abolition of the thyroid inhibition that normally follows emotional stress (restraint) though the inhibitory responses to laparotomy or injection of thyroxine are still retained

These results cannot be ascribed to the pituitary atrophy following stalk section since in the majority of cases very little if any such atrophy was observed to occur Two possibilities are raised by this work Either (*a*) all stimuli (restraint laparotomy and injection of thyroxine) act entirely through the nervous system and in the stalk cut animal laparotomy and thyroxine administration are still capable of causing liberation of sufficient chemical transmitter from the median eminence into the systemic circulation to affect the pituitary secretion of T S H or (*b*) so called neural stimuli (restraint) act entirely through the central nervous system and systemic stimuli (laparatomy, thyroxine) act additionally by producing a change in the chemical composition of the general blood stream which acts directly on anterior pituitary cells This latter view is thought to be the more likely

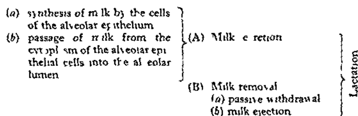
Localized electrical stimulation of the hypothalamus with observations of thyroid activity has rarely been studied Green and Harris (unpublished observations) stimulated various regions of the hypothalamus and pituitary gland of unanaesthetized rabbits for prolonged periods and tried to measure thyroid activity by studying the oxygen consumption of these animals No consistent change in oxygen consumption was obtained but the

tioned rats remained histologically normal (Uotila 1939*b*) that it reacted with the usual atrophy after injections of thyroxine or with the usual compensatory hypertrophy after subtotal thyroidectomy (Uotila 1940) but that the normal hypertrophy evoked by a cold environment was lacking (Uotila, 1939*b* and *c*) He suggested that the basic secretion of the thyrotrophic hormone is controlled by the blood level of thyroxine and is independent of the pituitary stalk but that under certain environmental conditions this rate of secretion can be modified by hypothalamic impulses which reach the hypophysis via the pituitary stalk Lack of good control procedures for checking the completeness of pituitary stalk section or hypophysial portal vessel regeneration however, leaves these results open to doubt Brolin (1945) recorded a decrease in thyroid activity following stalk section in the rat and agreed with Uotila that cold exposure of such animals does not lead to the usual hypertrophy He also found (Brolin 1947) that in the rat stalk section prevented the usual hypertrophy and vacuolization of pituitary basophile cells that occurs after thyroidectomy In the careful studies of Westman and Jacobsohn (1938) on the rat, and Westman Jacobsohn and Okkels (1942) on the rabbit, it was also found that pituitary stalk section results in the histological picture of diminished thyroid function In a more recent publication Barrnett and Greep (1951) found that stalk section in the rat resulted in thyroid atrophy and a reduced response to cold or administration of thiouracil Regeneration of the hypophysial portal vessels was stated to be absent However since stalk section was performed by the parapharyngeal approach and the ends of the cut stalk were found embedded in scar tissue it is possible that these results may be explained by some unobserved capillary anastomoses in the scar tissue sufficient to maintain a residual thyrotrophic hormone secretion [Such partial regeneration of the portal vessels has been observed to occur and was correlated with the occurrence of irregular and anovular oestrous cycles in stalk sectioned rats by Harris (1950)] Brown Grant Harris and Reichlin (unpublished—see Harris 1955) have lately studied the activity of the thyroid gland in rabbits in which the pituitary stalk had been sectioned In a group of rabbits the stalk was sectioned and a waxed paper plate inserted between the hypothalamus and pituitary gland In the rabbit however the supra

pituitary stalk section and pituitary transplants would indicate that the part of the central nervous system involved is the hypothalamus acting via the hypophysial portal vessels of the pituitary stalk

LACTOGENIC HORMONE
(prolactin luteotrophin)

The pituitary gland is related to the breast in two separate and distinct ways The anterior lobe secretes the lactogenic hormone which is concerned in the post parturient animal with the *secretion of milk* by the alveolar cells of the breast and the posterior lobe secretes the oxytocic hormone which is concerned with the reflex removal of milk known as *milk ejection* The different phases of lactation are illustrated in the scheme originally proposed by Dr Folley and extended by Cowie, Folley Cross Harris Jacobsohn and Richardson (1951)



It is with the milk secretion process that the anterior pituitary is functionally related and with the milk removal process that the posterior pituitary is related The double relationship must be borne in mind in considering experimental data for in the total lactational performance of an animal they are probably closely linked For example it is well known that complete and regular emptying of the mammary gland is an effective stimulus to secretion of milk Therefore if some experimental procedure interferes with the milk ejection process it may in turn result in a diminished rate of milk secretion

The data available at the moment indicate that the suckling stimulus by which is meant the sum of stimuli applied to the maternal animal by the activity of the young in their aim of obtaining milk from the mammary glands is also related to both milk secretion and milk removal Fig 46 illustrates the mechanisms which probably underly the effect exerted by suckling on

limitations of this method for measuring metabolic rate prevented any definite conclusions from being drawn Colfer (1949) found histological signs of increased thyroid activity in rats and rabbits following electrical stimulation of the hypothalamus providing such stimulation was of sufficient duration of at least four 1 hour periods on each of 2 days Stimulation was performed by means of implanted coils (remote control method) or by implanted electrodes with leads coming through the scalp No optimum site in the hypothalamus was found but control stimulation of the thalamus or corpus callosum was negative An increased dis charge of T S H from the pituitary gland has been reported by Saxton and Greene (1942) to follow the reflex stimulus to the gland of coitus in the rabbit and by Ellis and Wiersma (1945) to follow repeated electronarcosis in dogs and guinea pigs

It is important that the effects of localized hypothalamic stimulation should be studied in detail using the more sensitive methods now available for measuring thyroid activity, and the results compared with those obtained after placing hypothalamic lesions In studying the hypothalamic control exerted over a particular function such as T S H secretion and thyroid acti vity more reliable results are likely to be obtained from experi ments involving electrical stimulation than from tho e involving the placement of lesions The effects of a lesion may be wide-spread in the metabolic and nutritional spheres and may thereby produce atrophic endocrine changes that may not be *directly* related to the nervous structures damaged If electrical stimula tion of the site is found to produce a positive response the reverse of that produced by damage the evidence is very greatly strengthened that the neural structure exerts a direct influence over the function studied

At the present time the evidence shows that changes in the external environment usually referred to as stresses (except a lowered environmental temperature) result in rapid and marked inhibition of T S H secretion and of thyroid activity It is poss ible that future work will demonstrate this to be as uniform a reaction on the part of the thyroid gland as increased activity is on the part of the adrenal gland to the same stimuli That stress stimuli act at least in part through the central nervous system is clearly shown by the inhibitory responses of the thyroid fol lowing emotional stresses Studies of hypothalamic lesions,

(*b*) *The sensory stimulus of suckling and lactogenic hormone stimulation* Selye (1934) was the first to draw attention to the possibility that the nervous stimulation of suckling might be of importance in maintaining secretion of the lactogenic hormone and thereby milk secretion He showed that suckling may maintain milk secretion in the mammary glands of lactating rats even though removal of milk from these glands is prevented by tying the galactophores Further he demonstrated that suckling at some nipples would prevent involution in glands from which the nipple had been removed thus rendering suckling and milk removal impossible Selye and McKeown (1934*a*) in a further investigation found that involution of lactating mammary tissue finally occurs despite continued suckling if milk removal is prevented It seems likely therefore that under natural conditions the cessation of milk formation at weaning may be dependent on both cessation of suckling and lack of milk removal Perhaps a more striking demonstration of the effect of suckling on pituitary secretion since it differentiates between the effects of suckling and milk removal was afforded by Selye and McKeown (1934*b*) when they found that the regular oestrous cycle of rats and mice could be interrupted by supplying them with young litters Suckling occurred and resulted in great prolongation of the oestrous cycles maintenance of functional corpora lutea and full development of the mammary glands to lactation Since the conditions resembled closely that of pseudopregnancy following sterile coitus it was termed suckling pseudopregnancy These data and the more recent findings that the lactogenic hormone in the rat exerts a marked trophic effect on corpora lutea and is now sometimes referred to as the luteotrophic hormone affords strong support for the view that suckling in some ways stimulates the secretion of the lactogenic hormone by the pituitary gland This view is discussed fully by Selye Collip and Thomson (1934) The fact that the necessary element of suckling involved is the sensory stimulus to the nipple is indicated by the work of Hooker and Williams (1940) who demonstrated that the involution of mammary glands of lactating mice from which the litter was removed could be retarded by painting the nipples with turpentine Such irritation of the nipple was effective in preventing involution of the gland so painted and of other glands in the same animal

both milk secretion and milk ejection The possible control exerted by the hypothalamus over the secretion of the lactogenic hormone is the main concern here and the role of the hypo thalamus and posterior pituitary gland in milk removal will be considered later (Chapter 10)

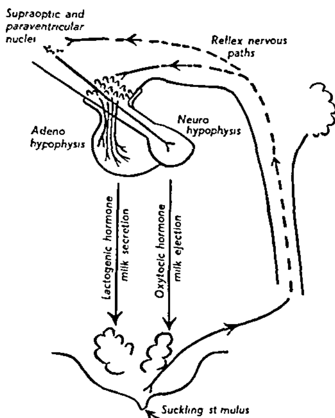


Fig 46 To illustrate the two probable neuro hormonal reflexes underlying *the stimulating action of suckling on milk secret on and milk ejection*

(*a*) *Motor nerves and the mammary gland* The fact that lactation can occur in the completely sympathectomized cat (Cannon Newton Bright Menkin and Moore 19-9) and in transplanted mammary glands providing normal glands are being suckled (Stricker 1929) demonstrates clearly that a motor nerve supply to mammary tissue is not involved in the process of lactation

(*b*) *The sensory stimulus of suckling and lactogenic hormone stimulation* Selye (1934) was the first to draw attention to the possibility that the nervous stimulation of suckling might be of importance in maintaining secretion of the lactogenic hormone and thereby milk secretion He showed that suckling may maintain milk secretion in the mammary glands of lactating rats even though removal of milk from these glands is prevented by tying the galactophores Further he demonstrated that suckling at some nipples would prevent involution in glands from which the nipple had been removed thus rendering suckling and milk removal impossible Selye and McKeown (1934*a*) in a further investigation found that involution of lactating mammary tissue finally occurs despite continued suckling if milk removal is prevented It seems likely therefore that under natural conditions the cessation of milk formation at weaning may be dependent on both cessation of suckling and lack of milk removal Perhaps a more striking demonstration of the effect of suckling on pituitary secretion since it differentiates between the effects of suckling and milk removal was afforded by Selye and McKeown (1934*b*) when they found that the regular oestrous cycle of rats and mice could be interrupted by supplying them with young litters Suckling occurred and resulted in great prolongation of the oestrous cycles maintenance of functional corpora lutea and full development of the mammary glands to lactation Since the conditions resembled closely that of pseudopregnancy following sterile coitus it was termed suckling pseudopregnancy These data and the more recent findings that the lactogenic hormone in the rat exerts a marked trophic effect on corpora lutea and is now sometimes referred to as the luteotrophic hormone affords strong support for the view that suckling in some ways stimulates the secretion of the lactogenic hormone by the pituitary gland This view is discussed fully by Selye Collip and Thomson (1934) The fact that the necessary element of suckling involved is the sensory stimulus to the nipple is indicated by the work of Hooker and Williams (1940) who demonstrated that the involution of mammary glands of lactating mice from which the litter was removed could be retarded by painting the nipples with turpentine Such irritation of the nipple was effective in preventing involution of the gland so painted and of other glands in the same animal

(*c*) *The central pathway of the suckling stimulus* The sensory path of the reflex excited by suckling has received little attention It would appear to consist of nerve fibres passing by a spinal route according to the findings of Ingelbrecht (1935) This worker found that severance of the spinal cord in lactating rats between the last dorsal and first lumbar segments resulted in failure of milk secretion and death of the young if only the posterior anaesthetic nipples were suckled whereas if suckling of the anterior nipples was allowed milk secretion was maintained in all glands The observation that lactation may follow extensive operations involving the thoracic wall (thoracoplasty and pneumonectomy) in non puerperal women of reproductive age (Salkin Hopement and Davis 1949) and that gynecomastia occurred in 7 out of 32 men with traumatic injuries of the spinal cord (Cooper and Hoen 1949) might also be explained on the grounds of traumatic irritation of sensory fibres in the intercostal nerves or spinal tracts *It is likely that the more central pathways* involve the hypothalamus and pituitary stalk The evidence for this statement is derived from observations of the effects of pituitary stalk section and pituitary transplantation The results obtained by different workers following stalk section have been as divergent in this field as those in which other anterior pituitary functions have been studied Herold (1939) and Desclin (1940) claim that section of the pituitary stalk in rats causes failure of lactation in spite of functioning anterior pituitary tissue and continued suckling Jacobsohn and Westman (1945) found mammary involution occurred in lactating rats following this operation but that it was not so severe as that which followed hypophysectomy whilst Dempsey and Uotila (1940) observed that stalk transected rats may lactate normally Looking back at this earlier work however it is seen that there are two variable factors which may have influenced the results and were not controlled (*a*) regeneration of the hypophysial portal vessels across the site of the stalk section and (*b*) failure of oxytocic secretion by the neurohypophysis resulting in failure of milk ejection and thereby indirectly resulting in *failure of milk secretion At the* time of the later work of Harris and Jacobsohn (1952) more was known about these two factors and control steps were taken In these experiments hypophysectomized rats were grafted with pituitary tissue in the subarachnoid space under the temporal

lobe of the brain and under the median eminence of the hypothalamus As described previously these grafts became richly vascularized and about the same amount of tissue was anatomically well maintained in both sites Animals with grafts under the temporal lobe of the brain remained persistently anoestrous and showed atrophy of the reproductive organs so that no observations were possible in the lactational performance of these animals However rats with median eminence grafts returned to normal oestrous rhythm Six of these animals after placing with males became pregnant and delivered living normally developed young at term After parturition the maternal mammary glands of the 6 rats were found to be well developed the alveoli and ducts being distended with secretion In spite of vigorous suckling however the young did not obtain the milk present in the mammary glands and died from starvation It was found that this could be prevented by subcutaneous injections of oxytocin into the maternal rat After such an injection a copious transfer of milk occurred from the mother to the litter a process which was easily and repeatedly observed through the transparent abdominal walls of the young The conclusion drawn from these experiments were that the nerve fibres of the pituitary stalk are necessary for the reflex release of oxytocin and for normal milk ejection but are not necessary for the release of the lactogenic hormone and for milk secretion It is likely that the suckling stimulus excites secretion of the lactogenic hormone by means of a pathway involving the hypophysial portal vessels a view compatible with the above results but not established by them since the control animals failed to become oestrous and their lactational performance was not studied

In summary it may be said that the view that suckling stimulates the secretion of the lactogenic hormone by means of a nervous reflex passing probably through the hypothalamus offers an explanation of many data that are otherwise difficult to understand In the above account the term lactogenic hormone has been used throughout but it may be well to bear in mind as pointed out by Folley and Young (1941) that lactogenesis is probably a response to the co ordinated actions of a complex of anterior pituitary hormones centring around the lactogenic hormone as an essential element In this case it would seem probable that the physiological stimulus of suckling causes the secre

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tion of a particular pattern of hormones The word hormones is here used in the biochemical sense of preparations containing single activities

GROWTH HORMONE
(diabetogenic factor, somatotrophin)

Strong evidence has recently been put forward that diabetogenic activity is an inherent property of growth hormone (Reid, 1952) Although few studies have been made of any control exerted by the hypothalamus over the secretion of the growth hormone two lines of evidence indicate that such control may occur

(i) Nearly all workers agree that transplantation of the pituitary gland to a site of the body remote from the sella turcica leads to marked retardation of body growth (see Chapter 3)

(ii) The cardinal signs of interference with carbohydrate metabolism that follow hypophysectomy have been found to follow the placement of various hypothalamic lesions mainly in the region of the paraventricular nucleus (for review of this subject see Harris 1948) Spirtos Bogdanove and Halmi (1954) have recently found that the increased insulin sensitivity of hypophysectomized rats or of rats in which electrolytic lesions have been placed in the hypothalamus may be reduced to normal by administration of somatotrophin

Weininger (1954) has found that rats subjected to handling and petting for 3 weeks after weaning (days 23–44 of life) show substantial increase in growth and resistance to stress as compared with control litter mates There was no indication in these experiments as to the means by which the growth rate was accelerated but the observations do seem to offer a possible approach to an analysis of the factors that may be involved in the secretion of growth hormone

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by direct microscopic observation of the vessels in amphibians (Green 1947) and in the rat (Green and Harris 1949) Studies in which the structures in this region were submitted to electrical stimulation revealed that stimulation of the hypothalamus may evoke gonadotrophic (Markee Sawyer and Hollinshead 1946 Harris 1948) or adrenocorticotrophic (de Groot and Harris 1950) secretion from the adenohypophysis whereas direct stimulation of the gland itself was ineffective This finding is compatible with the view that the gland is humorally excited and argues against the view of a secreto motor innervation to the gland When it was found that the portal vessels may regenerate quickly after pituitary stalk section and that the gonadotrophic activity of the anterior pituitary after this operation may be correlated with such vascular regeneration (Harris 1950) it became clear that the remarkably discordant findings of different workers regarding anterior pituitary function after section of the stalk might be explained by varying degrees of regeneration of the vessels Regeneration of these vessels has now been observed in mice (de Groot 1952) rats (Harris 1950 Harris and Jacobsohn 1950) rabbits (Jacobsohn personal communication Brown Grant Harris and Reichlin unpublished) ferrets (Thomson and Zuckerman 1953 Donovan and Harris 1954) and monkeys (Harris and Johnson 1950) Finally a survey of the literature reveals that when the pituitary gland is transplanted to a site in the body remote from the sella turcica only fragments of normal function at most are to be observed However when anterior pituitary tissue is grafted under the hypothalamus it becomes revascularized by the portal system of vessels and is capable of maintaining normal functional activity (Harris and Jacobsohn 1952)

From the data reviewed above there can be little doubt that vascularization of anterior pituitary tissue by the hypophysial portal system is necessary for the maintenance and control of normal activity of this gland So much can be taken as established The question as to how a portal blood supply exerts such a specific effect on anterior lobe tissue is debatable and forms a most difficult and intriguing problem There are clearly many possibilities but the most likely seems to be that nerve fibres from the hypothalamus liberate some humoral substance(s) into the capillaries of the primary plexus in the median eminence and

CHAPTER 7

NEUROVASCULAR CONTROL OF THE ADENOHYPOPHYSIS

General data

Over twenty years ago Hinsey and Markee (1933) stated or the pathways from the hypothalamus must activate the posterior lobe of the hypophysis which in turn may exert an influence on the anterior lobe by hormonal transmission Friedgood (1936) *suggested that the anterior pituitary might be stimulated either* by the liberation of an adrenergic substance at the terminals of sympathetic fibres in the gland or through the secretion within the hypothalamus of sympathin or a similar adrenergic substance which is then carried to the gland through the portal circulation of the pituitary stalk Harris (1937) and Brooks (1938) both mentioned rather tentatively the possibility that a stimulus might be humorally transmitted from the hypothalamus or neurohypophysis to the adenohypophysis It is of interest that at this early time workers were being rather forced to this possibility to afford an explanation of the facts that (*a*) the hypothalamus forms part of a nervous reflex path by which the stimulus of coitus excites the secretion of gonadotrophic hormone from the pars distalis of the rabbit and (*b*) the anterior lobe of the pituitary has at most a very scanty nerve supply In the following years little attention was paid to the hypophysial portal system as a possible control path of anterior pituitary function probably because the direction in which the blood flowed in these vessels was far from clear In 1941 however, Taubenhaus and Soskin carried the general idea a step further and from their work on rats suggested that the hypophysial portal vessels might carry an acetylcholine-like substance from the hypothalamus to the anterior pituitary The possibility of humoral transmission was considered in more detail and the evidence regarding it summarized by Harris (1944) and after a further study of the anatomy *of the vessels was restated by Green and Harris (1947)* The direction of blood flow in the portal vessels was then established as being from the tuber cinereum to the adenohypophysis

The time taken for blood to flow from the primary plexus in the median eminence to the sinusoids in the anterior lobe of the pituitary is difficult to estimate From direct observation of such blood flow in these vessels of the rat a very rough approximation might be given as 1 second On these grounds it would seem unlikely that a substance such as acetylcholine that is quickly broken down in the blood stream would be a likely candidate for the role of such a humoral transmitter

On microanatomical grounds it has been suggested (Benoit and Assenmacher 1953 Palay 1953 Rothballer 1953) that the so called *neurosecretory material* present in the nerve tracts passing from the supraoptic and paraventricular nuclei into the neuro hypophysis may be liberated into the portal vessels and play a role in regulating the activity of the anterior pituitary The nerve fibres which contain this material are certainly intimately related to the primary plexus of the portal vessels but the fact remains that electrical stimulation of these nerve fibres does not neces sarily result in increased gonadotrophic (Harris 1948) or adreno corticotrophic (de Groot and Harris 1950) secretion The relationship between the neurosecretory material and the pos terior pituitary gland is discussed in Chapter 11

(*b*) *Analysis of hypothalamic extracts* The hypothalamus has been assayed for various substances of known biological activity and the content of such substances in the hypothalamus and in the median eminence compared with the content in other parts of the brain In respect to humoral transmission in the portal vessels one difficulty arises here There is no reason to believe that any transmitter substance would be stored in the median eminence rather than formed and immediately released into the blood stream Therefore the absence of any particular substance in extracts of the median eminence does not eliminate the possi bility that the compound plays an active part in humoral trans-mission of stimuli to the pituitary gland

Feldberg and Vogt (1948) measured the content of the enzyme or enzyme system which forms acetylcholine (rather than pre-formed acetylcholine) in different parts of the central nervous system of the dog The infundibulum presumably including the median eminence was found to possess little power of acetyl choline synthesis

Vogt (1954) studied the distribution of sympathomimetic

that this substance is carried by the portal vessels to excite or inhibit the cells of the pars distalis

Data regarding the chemical nature of possible neuro vascular agents

(*a*) *Anatomical* Some information regarding the probable nature of any chemo transmitter may be obtained by a study of the anatomical arrangement of the structures that would be involved in its transport

The origin of the substance appears to be from nerve fibres and not nerve cells The primary plexus of capillaries which form the origin of the hypophysial portal vessels lies in the median eminence of the tuber cinereum and in some forms in the infundibular stem and no discrete group of cells can be found related to this plexus The scattered cells present in the median eminence do not show any obvious or intimate relation ship with the vessels On the other hand a multitude of nerve fibres come into immediate juxtaposition with these thin walled capillaries

In order to pass from nerve terminals into the blood stream the substance would have to possess a certain diffusibility which would seem to limit its molecular size In most animals the walls of the vessels of the primary plexus consist of little more than a layer of endothelium However in man Green (1948) states that the same vessels have a coat of collagen reticular fibres and poss ibly smooth muscle cells This same worker also describes a variety of nerve terminals within the coats of these vessels such terminals he states are common An illuminating example in this respect is shown by the bird (Wingstrand 1951) In this form the primary plexus of the portal vessels forms a dense net work on the surface of the median eminence In large birds such as the goose the surface of the median eminence is corru gated and the vessels of the plexus lie in the furrows Wingstrand describes nerve fibres from the hypophysial tract running ver tically to the surface of the median eminence forming loops there and then retracing their course deeply A chemical sub stance liberated by these nerve fibres would have to diffuse through a thin reticular membrane the endothelium of the vessels and possibly a glial sheath This fact would again indicate that the hypothetical chemo transmitter is of low molecular weight

years the results obtained are of doubtful significance for it is now known that non specific trauma (Friedgood and Bevin 1941 Swingle Fedor Barlow Collins and Perlmutt 1951) certain anaesthetics (Swingle et al 1951) and injection of a wide variety of substances (Swingle Seay Perlmutt Collins Barlow and Fedor 1951) may result in a pseudopregnancy response in a high proportion of rats It seems probable that nervous or stressful procedures of widely different kinds may elicit luteotrophic secretion and a state of pseudopregnancy in the rat

The release of luteinizing hormone from the anterior pituitary of the rabbit with resultant ovulation has been studied by Markee and his co workers Markee Sawyer and Hollinshead (1948) reported that injection of adrenaline into the pituitary gland by a parapharyngeal approach resulted in ovulation in a proportion of cases The animals received three doses of about 40 μl each in the course of 30 minutes and it was found that a strength of 1/1 000 adrenaline hydrochloride was the most effective (as compared with strengths of 1/100 1/10 000 and 1/50 000) and produced positive results in 5 out of 10 rabbits The total dose administered over the 30 minutes was therefore about 100 μg expressed as adrenaline Control injections were made using potassium cyanide in physiological saline and an acetyl choline eserine mixture and in these cases only 1 out of 23 animals showed any sign of ovarian activation This work is of much interest but the conclusion that Markee et al have more recently drawn from this and other evidence that some adrenergic substance is the humoral transmitter carried by the portal vessels and responsible for the release of the luteinizing hormone in the rabbit has been criticized on various grounds Firstly the dose of adrenaline necessary to produce ovulation administered in this way seems very large However the amount of anterior pituitary tissue that the injected solution reaches may form only a small proportion of the total gland Secondly the pituitary is composed of soft friable tissue and is enclosed in a firm fibrous tissue capsule therefore any injected solution may damage the gland and so result in release of stored hormone into the circulation The injection of control solutions without ovulation would appear to negate this criticism This work has recently been repeated using another technique (Donovan and Harris unpublished) Instead of using an open operative approach to the

amines in the central nervous system and found the highest concentration of these substances to be in the hypothalamus mid brain and medulla Noradrenaline was found in greater quantity than adrenaline about 1 μg per gram of fresh tissue being present in the hypothalamus The presence of fairly large quantities in the midbrain makes it difficult to correlate the occurrence of this substance in the hypothalamus with any function related to the hypophysial portal vessels It is of interest that noradrenaline has also been found in an astrocytoma composed mainly of glial elements in higher concentration (7 5 μg/gm tissue) (Bulbring Philpot and Bosanquet, 1953)

The histamine content of various parts of the brain with special reference to the hypothalamo hypophysial region has also been assayed (Harris Jacobsohn and Kahlson 1952) in the cat dog and pig Confirming previous workers the posterior lobe of the pituitary was found to contain histamine in high concentrations The anterior lobe was also estimated to be rich in this substance The hypothalamus as a general trend contained approximately the same amount as sympathetic ganglia though the median eminence itself was found to be conspicuously rich in histamine The total brain and other parts of the brain such as the thalamus, nucleus caudatus and cerebellum contained very small amounts

These studies make it seem unlikely that acetylcholine plays any part as a humoral transmitter in the hypophysial portal vessels but leaves the question open for sympathomimetic amines and histamine

(c) *Stimulation of anterior pituitary secretion by experimentally administered substances* The effect of various substances in exciting anterior pituitary tissue to secretory activity has been investigated with the gland in its normal position and after transplantation of the gland to a distant site

(i) *Gland in normal position* Taubenhaus and Soskin (1941) were among the first workers to investigate the effect of local application of substances to the pituitary gland They applied an acetylcholine prostigmine mixture to the pituitary gland of oestrous rats under avertin anaesthesia the gland being exposed surgically by a parapharyngeal route They found that 13 out of 20 rats so treated became pseudopregnant Although this work was probably responsible for stimulating similar studies in later

effect on the ovaries A rough calculation from the data given by Sawyer et al (1950) shows that their intravenous dose represents a delivery of about 2–20 μg adrenaline per minute to the anterior pituitary gland and although little reliance may be placed on this figure it appears to be in the approximate range of the dose necessary to produce ovulation by direct injection in the gland

The large amount of adrenaline necessary to evoke the release of luteinizing hormone from the rabbit s anterior pituitary gland is compatible with the view that the hormonal substance under lying such secretory activity is adrenergic in nature but is not adrenaline Few data are available regarding the activity of other adrenergic substances Donovan and Harris (unpublished) found that injection of *l* noradrenaline directly into the pituitary was ineffective in causing ovulation in rabbits

The effect of systemically administered adrenaline in causing increased or decreased secretion of other anterior pituitary hor mones has been investigated in detail for A C T H and some information is also available for T S H The significance of these results however may be different from those regarding the release of luteinizing hormone Firstly the doses of adrenaline necessary to evoke release of A C T H are very much smaller and are in the range of the amounts liberated by the animal s own adrenal medulla and secondly an increased secretion of A C T H is evoked by many different types of trauma and by the administration of innumerable substances so that the significance of results obtained by applying any particular substance is diffi cult to assess Long and his co workers (Long 1947 Gershberg Fry Brobeck and Long 1950) have made a detailed study of the effects of adrenaline on A C T H secretion They suggest that the initial increase in A C T H secretion caused by stress is due to the adrenal medulla producing an increased systemic blood level of adrenaline and that endogenous adrenaline is the specific excitant of anterior pituitary cells in this reaction It is clear however that equally rapid reactions may occur in animals after adrenal demedullation (see Hodges 1953 and others) so that if adrenaline is the underlying factor in this response it is being derived from some tissue other than the adrenal medulla Long (1951) has discussed the possibility that adrenaline may be a humoral agent carried by the portal vessels If this is so then both an increased systemic or hypophysial portal blood content

gland the rabbits heads were orientated in a stereotaxic instrument and a fine hollow needle passed through a small drill hole in the vault of the skull into the pituitary gland Injection was made at the rate of 0 2 μl per minute for 50 or 100 minutes thus approaching more closely to the conditions of a continuous infusion Solutions of adrenaline bitartrate were used and it was found that doses (expressed as adrenaline) of 66 μg resulted in positive responses in 1 out of 4 rabbits and of 33 μg in 2 out of 9 animals Control injections of noradrenaline bitartrate were negative From these results it was felt that further improvement in the technique so that the injected adrenaline was distributed evenly throughout the anterior lobe might result in more significant results To obtain this end injections were made into the region of the median eminence on the grounds that the solution might diffuse into the primary plexus of the portal vessels and thereby be rapidly and widely distributed to the anterior pituitary gland That such might occur was established by killing animals 10–30 seconds after injection of diffusible dyes in this site and examining the distribution of the coloured material Injection of adrenaline bitartrate solution unneutralized or roughly neutralized with indicators at the same rate as used for the injections into the pituitary gland and in a dose of 66 μg (adrenaline) resulted in ovarian activation in 12 out of 24 rabbits When the solution was adjusted carefully to a pH of 6 80–7 40 the same or larger dose (to allow for decomposition of the adrenaline at neutral pH) gave a positive response in only 2 out of 13 rabbits This evidence together with the fact that similar injections of weak solution of ammonium tartrate also resulted in ovulation in two out of five experiments indicates that injection of substances into the tuber cinereum may result in ovulation by chemical or physical stimulation of nerve fibres It would be dangerous then to draw conclusions regarding the nature of any humoral transmitter from data derived from injections of solutions into the region of the hypothalamus

In later work Sawyer Markee and Everett (1950a) found that intravenous injection of adrenaline (1 mg/kg) into rabbits protected against the lethal effects of such doses of adrenaline by previous administration of atropine resulted in ovulation in five out of seven cases Other workers using unprotected rabbits and smaller doses of intravenous adrenaline had failed to find any

eminence directly to the transplanted anterior pituitary gland and observing any stimulated release of hormones has not yet been performed

(d) Pharmacological blockade of the reflex release of anterior pituitary hormones This line of approach has been used to investigate the mechanism controlling release of the gonadotrophic and adrenocorticotrophic hormones

Following the observation that injection of adrenaline in large doses into the pituitary gland results in ovulation in the rabbit (Markee Sawyer and Hollinshead 1948) Sawyer Markee and Hollinshead (1947) found that the administration of dibenamine a sympatholytic drug within one minute from copulation prevented ovulation in 16 out of 19 rabbits Everett Sawyer and Markee (1949) extended these observations to the rat and found that in this spontaneously ovulating form dibenamine also blocked release of luteinizing hormone However Nickerson (1949) criticized these experiments on the grounds that dibenamine has an early excitant action on the central nervous system and a later adrenergic blocking action Maximum blockade he pointed out does not occur until 1½ hours after intravenous administration and since Markee and his co workers found it necessary to administer dibenamine within one minute of copulation for ovulation to be blocked and since in their hands administration was regularly followed by signs of extreme central nervous system stimulation Nickerson suggested the results obtained might have been due to the central excitatory properties of dibenamine It may be further argued that the stress revealed by the opisthotonus convulsions and loss of body weight which followed injection of dibenamine was sufficient to block L H release The findings of Dordoni and Timiras (1952) that injection of adrenaline eserine or formalin or spinal cord transection block oestrogen induced ovulation in 40 per cent of pregnant rats may be quoted in support of this view However Sawyer Markee and Everett (1949) later reported control experiments in which it was found that dibenzylaminoethanol a hydrolysis product of dibenamine (which retains the central excitatory action but which is non adrenolytic) did not block L H release from the rabbit or rat pituitary Further work with the less toxic drug SKF 501 also indicated that the pituitary blockade was due to the sympatholytic properties of the compound (Sawyer Markee and Ever

of adrenaline could activate A C T H discharge and many difficulties in our present views could be explained It may be mentioned here that intravenous injection of *l* noradrenaline is less effective than *l* adrenaline in causing A C T H discharge (Nasmyth 1950)

Adrenaline administered systemically will cause not only an increase in A C T H release but a decreased thyroid activity which probably reflects a decreased rate of release of T S H (Brown Grant Harris and Reichlin 1954) The doses necessary to produce thyroid inhibition however are large (250 μg s c twice daily to a rabbit Brown Grant et al 1954) and it seems likely that the adrenaline in these experiments is acting only as a stressing agent

Slusher and Roberts (1954) have recently produced evidence that a lipide or lipoprotein present in extracts of the posterior hypothalamus but not in extracts of the anterior hypothalamus or cerebral cortex may be concerned in the stimulation of A C T H secretion by the adenohypophysis

(ii) *Transplanted gland* In order to obtain more direct evidence regarding the effect of various substances on pituitary cells several workers have used the hypophysectomized animal in which the pituitary gland has been transplanted usually to the anterior chamber of the eye Different substances have then been administered into the systemic circulation or locally in the vicinity of the graft and the effect on secretion of A C T H studied This method of utilizing pituitary transplants has the advantage of localizing the site of action of an administered substance more clearly McDermott Fry Brobeck and Long (1950) studied the effect of adrenaline in releasing A C T H from pituitary tissue transplanted to the anterior chamber of the eye of hypophysectomized rats They found that both subcutaneous injection or adrenaline *or injection into the eye bearing the transplant* resulted in release of A C T H as demonstrated by an eosinopenia The significance of this result is rendered doubtful however by the observation of Fortier (1952) that not only adrenaline but also histamine will provoke A C T H release from such transplants *Fortier applied these drugs by subconjunctival injection* in four different sites thus diminishing the risk that the injection might provoke a response by some physical effect on the graft such as mechanical pressure or a change in temperature

The interesting experiment of applying extracts of the median

blocking agents ergotamine dibenamine and TEA in the doses they used all lower the adrenal ascorbic acid content of rats Following ergotamine and dibenamine administration a further fall was observed subsequent to epinephrine injection or various forms of stress After TEA injection the adrenal ascorbic acid depletion was not increased by stress Other workers (Paschkis Cantarow Walkling and Boyle 1950 Fortier 1950 Sawyer and Parkerson 1953) have conducted similar experiments and the general conclusion is that sympathetic blocking drugs do not abolish although they may diminish the A C T H discharge caused by stress

It seems unlikely that a cholinergic mechanism is concerned in the regulation of A C T H secretion Dordoni and Fortier (1950) showed that the adrenal ascorbic acid depletion following administration of eserine is increased by the simultaneous ad ministration of atropine Atropine alone was found to produce a significant fall in adrenal ascorbic acid that was prevented by previous hypophysectomy

In view of the fact that the blocking agents themselves produce A C T H release a feature which is probably related to the fact that they are to some extent toxic compounds it is difficult to draw definite conclusions from the above work The procedure of Guillemin and Fortier (1953) however seems likely to give clearer results when using this approach These workers investigated the possibility of A C T H release being controlled by a histaminergic mechanism They utilized the antihistamine drug phenergan for this purpose and showed that chronic administra tion of phenergan (for 10 days) led to a state of adaptation *In* this state injection of phenergan or phenergan plus histamine had no effect on the ascorbic acid content of the rat s adrenal although other stresses such as cold sound and immobilization resulted in the usual A C T H discharge and adrenal ascorbic acid de pletion Guillemin (personal communication) has more recently used rats adapted to the non specific effects of atropin SKF 501 and dibenzyline and found that the usual fall in adrenal ascorbic acid follows injection of formalin or immobilization although the effects of injecting acetyl β methyl choline adrenaline and nor adrenaline were abolished The only pharmacological agent that has been reported to block A C T H release in response to stress is morphine (Briggs and Munson 1954)

ett 1950*b*) The fact that atropine also blocks pituitary release of L H in the rabbit providing it is injected within the first few seconds after copulation (Sawyer Markee and Townsend 1949) may indicate a cholinergic link in the nervous reflex path by which the pituitary is stimulated or may indicate that the inhibition of L H release is relatively non specific

Since the timing of ovulation is of much interest in agricultural circles the question of pharmacological control of L H release and ovulation in farm animals has attracted attention It has been found that atropine will delay the time of ovulation in heifers by 24-66 hours (Hansel and Trimberger, 1951) and that atropine and dibenamine will block ovulation in the hen (Van Tienhoven Nalbandov and Norton 1954) However the interruption of ovulation in the hen has been reported to follow subcutaneous injection of non specific substances (ovalbumin, casein peptone) which possibly act in virtue of some toxic effects produced (Fraps and Neher 1945)

Blocking drugs have also been used to investigate the possibility of an adrenergic cholinergic or histaminergic link in the sequence of events by which a stress stimulus evokes secretion of A C T H The position here however is more complicated for the injection of the blocking agent itself is sufficient stress to evoke A C T H discharge before the specific blockading action is developed

The possibility of an adrenergic mechanism participating in the release of A C T H following stress has been investigated by employing drugs which block transmission in autonomic ganglia (tetra ethyl ammonium chloride TEA) or which block the effects of postganglionic sympathetic stimulation and of exogenous adrenaline (dibenamine SKF 501) Tepperman and Bogardus (1948) found that although the sympathetic blocking action of dibenamine persists for several days after a single dose the adrenal ascorbic acid depletion produced by the dibenamine lasted only 10 hours They accordingly subjected rats to the stress of intraperitoneal injection of carbon tetrachloride 24 hours after the administration of dibenamine but found no inhibition of the adrenal response to the stress Administration of TEA alone produced no fall in adrenal ascorbic acid and did not inhibit the adrenal response to stress A more extensive investigation by Ronzoni and Reichlin (1950) revealed that the adrenergic

physectomized female rat would support normal female reproductive activity such as the rhythm of oestrous cycles pregnancy and lactogenesis Since it is unlikely that the pituitary gland of the male normally secretes in a manner necessary to maintain such activity it may be concluded that the hypothalamus supplies not only a general stimulus to anterior pituitary function but also sets the pattern of this function

A second question then arises If the hypothalamus acting via the hypophysial portal vessels regulates the rate of secretion of the anterior pituitary hormones are there as many humoral mechanisms involved as there are hormones? This question in turn raises the problem of how many hormones are separately released by the anterior pituitary gland into the blood stream At least six purified fractions have been obtained from anterior pituitary extracts but the evidence is meagre that they constitute hormones in the strict sense The general view seems to be that these extracts if not the actual hormones represent the activities of hormones that may be secreted separately (see discussion at Josiah Macy (Junr) Conference on the adrenal cortex 1951) The evidence for this belief lies in the findings that certain stimuli such as physical trauma cold and various sexual stimuli appear to evoke specific effects on individual target glands (adrenal cortex thyroid and ovaries respectively) Further injection of a single target organ hormone such as an oestrogen seems to result in atrophy of only the corresponding target organ i e the ovary More detailed studies however may reveal the need for extending these views As one example the recent finding of Brown Grant Harris and Reichlin (1954) that many different forms of stress stimuli inhibit thyroid activity may be quoted It was found that a variety of emotional and physical stress stimuli well known to produce A C T H release and adrenal cortical activation resulted in inhibition of the thyroid gland due to decreased secretion of T S H It seemed possible that under a wide variety of conditions the secretion of A C T H and T S H by the rabbit pituitary bear a reciprocal relationship one to the other However it is known that exposure to a cold environment excites thyroid activity and that exposure to a temperature of (about) 0 C acts as a stress to activate the adrenal cortex Cold could therefore be said to stimulate both T S H and A C T H secretion In the course of another study how

In summary it may be said that experiments involving the use of blockading drugs have not produced any convincing evidence of the participation of an adrenergic cholinergic or histaminergic agent in the control of gonadotrophic and adrenocorticotrophic hormone release from the adenohypophysis Although the evidence produced appears to be valid in relation to humoral excitation carried via the systemic or hypophysial portal circulation it is doubtful whether it is significant in considering humoral transmission across synapses in the central nervous system Before considering this latter point it would be necessary to establish the fact that the various blocking agents used have crossed the blood brain barrier in sufficient concentration to be effective at such synapses

General Discussion

There can be little doubt that the maintenance and regulation of the activity of the anterior pituitary gland is dependent on the gland being supplied with blood via the hypophysial portal vessels The information as to the details of the mechanism involved is scanty but it seems likely that nerve fibres in the hypothalamus liberate some humoral substance into the primary plexus of the vessels and that this substance is carried by the vessels to affect anterior pituitary activity

The above view raises further questions Firstly do the hypothalamus and the portal circulation supply only a general stimulus to anterior pituitary activity leaving the secretion of the individual trophic hormones to be regulated by other factors such as the level of the target organ hormones in the general blood stream? This is unlikely since certain stimuli such as coitus in the rabbit acting by reflex paths in the hypothalamus appear to elicit increased secretion of particular hormones (in this case the luteinizing hormone) However there is little exact information available as to whether other anterior pituitary hormones are discharged by the act of mating in the rabbit and indeed the study of Saxton and Greene (1942) indicates that such is the case More convincing evidence that the hypothalamus plays a part in regulating the detailed pattern of anterior pituitary secretion is supplied by the study of pituitary transplants (Harris and Jacobsohn 1952) In this work it was found that anterior pituitary tissue from a male rat placed under the stalk of a hypo

but for the present purpose they suffice to illustrate the need for more detailed studies of the hormonal secretion elicited from the anterior pituitary by various physiological stimuli The final technique by which such observations will be made is one involving direct measurement of the hormones in the venous blood from the pituitary gland Until such techniques are available however much valuable information could be obtained by *simultaneous* measurement of the changes elicited in the pituitary target organs of an animal by a particular stimulus

A further possibility to consider is that the discharge of different anterior pituitary hormones may be evoked by the same humoral stimulus but at different thresholds There is some evidence which would indirectly support this view Studies of the effect of pituitary stalk section and of pituitary transplants under the median eminence indicate that the first target organ to atrophy after stalk section and the last to regain normal size and function after pituitary transplants is the ovary It seems that less hypophysial portal vessel regeneration is required to maintain thyrotrophic and adrenocorticotrophic secretion than gonadotrophic One possible explanation is that the same humoral stimulus may be acting but at different levels of concentration

A final point for discussion concerns the histological localization of the various cell types in the pars distalis of the pituitary Fig 47 shows a diagram of a horizontal section through the gland There are several zones of the pars distalis distinguishable on the basis of the cell types Firstly the anterior pole of the gland the zona tuberalis which contains basophile and chromaphobe cells (Dawson 1937) This is the part which in the rabbit is separated from the rest of the pars distalis by connective tissue trabeculae and contains the trunks of the portal vessels The main mass of the pars distalis lying posterior to the zona tuberalis may be divided into different areas according to the preponderating cell types (Rasmussen 1938 Purves and Griesbach 1951) It seems possible that the zonal differentiation of cells is dependent on the vascular architecture of the gland If different cell types are dependent on a different humoral stimulus and if the liberation of humoral stimuli are regulated by different nerve fibre tracts in the hypothalamus a correlation might be drawn between medial and lateral nerve tracts in the

ever (Brown Grant von Euler Harris and Reichlin 1954) it was found that exposure to moderate degrees of cold (about 15° C) was a more effective stimulus to thyroid activity than exposure to more severe degrees of cold (1 C) which occasionally resulted in thyroid inhibition It is possible then that the

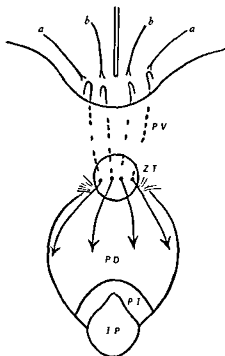


Fig 47 Diagram to illustrate the possibility that the histological differentiation of the central and lateral parts of the pars distalis (*P D*) of the pituitary may be determined by the vascular architecture of the portal vessels (*P V*) of the stalk whose functional activity may in turn be secondary to various nerve tracts in the hypothalamus (lateral tracts *a* medial tracts *b*) *Z T* zona tuberalis *P I* pars intermedia *I P* infundibular process

low temperature necessary to stimulate the adrenal cortex affects the thyroid gland as a non specific inhibitory stress Such a view has been expressed previously (Bogoroch and Timiras 1951) Although cold can be said in general to result in increased secretion of T S H and A C T H it is possible that these activities are elicited by different degrees of cold and do not occur simultaneously The above results require confirmation

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median eminence medial and lateral trunks of the portal vessels in the pituitary stalk and a medial and lateral grouping of cell types in the pars distalis There are good grounds for believing that the various portal trunks supply different areas of the pars distalis Partial sections of the pituitary stalk in rats followed by vascular perfusion with indian ink demonstrate a type of end artery relationship between the vessels and the different regions of the gland

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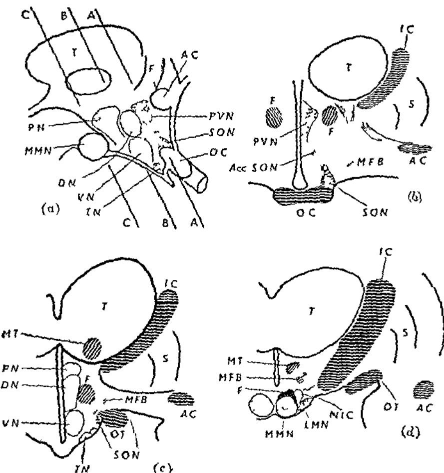


Fig 48 (a) Diagram of a sagittal section through the hypothalamus showing the hypothalamic nuclei related to the third ventricle (after Clark W E LeGros 1936 *J Anat Lond*)

(*b*) Diagram of a transverse section through the supraoptic region of the hypothalamus (plane *A–A* Fig 48 *a*)

(*c*) Diagram of a transverse section through the tuberal region of the hypothalamus (plane *B–B* Fig 48 *a*)

(d) Diagram of a transverse section through the mammillary region of the hypothalamus (plane *C–C* Fig 48 *a*)

A SON accessory supraoptic nucleus *AC* anterior commissure *DN* dorso medial hypothalamic nucleus *F* fornix *IC* internal capsule *LMN* lateral mammillary nucleus *MFB* medial forebrain bundle *MMN* medial mammillary nucleus *MT* mammillo thalamic tract *NIC* nucleus intercalatus *OC* optic chiasma *OT* optic tract *PN* posterior hypothalamic nucleus *PVN* paraventricular nucleus *S* striatum *SON* supraoptic nucleus *T* thalamus *TN* tuberal nuclei *VN* ventro-medial hypothalamic nucleus

CHAPTER 8

ANATOMY OF THE HYPOTHALAMUS THE NERVOUS AND BLOOD SUPPLY OF THE NEUROHYPOPHYSIS

Anatomy of the hypothalamus

Excellent reviews dealing with the anatomy of the hypothalamus have been published (Clark 1938 Ingram 1940) and reference should be made to these publications for detailed information concerning the embryology comparative anatomy blood supply and morphology of the hypothalamic area To become practically acquainted with the anatomy of the hypothalamus it may be found convenient to study serial sections through the brains of rats in conjunction with the papers of Gurdjian (1927) and Krieg (1932)

The hypothalamus is an ill defined structure Viewed from below it is bounded by the optic chiasma optic tracts posterior perforated substance and cerebral peduncles A midline sagittal section shows the hypothalamus to be separated from the thalamus by the hypothalamic sulcus on the wall of the third ventricle *Anteriorly it merges with the preoptic and septal* regions and posteriorly with the tegmental area of the midbrain On the lateral side lies the subthalamus and the internal capsule

On *examining horizontal sections through the hypothalamus* three great tracts of myelinated nerve fibres are immediately obvious These tracts are obliquely placed though their main direction is dorso ventral In order, from anterior to posterior, they are the fornix the mammillo thalamic tract (bundle of Vicq d Azyr) and the habenulo peduncular tract (fasc retroflexus of Meynert) Since these tracts run deeply in the wall of the third ventricle they divide each side of the hypothalamus into medial and lateral divisions In the lateral region runs the medial fore brain bundle The medial part of the hypothalamus is often divided into rostral or supraoptic tuberal and caudal or mammillary regions and contains the majority of the hypothalamic nuclei (Fig 48)

Nerve fibre connections of the hypothalamus

The fibre connections of the hypothalamus are carried in a few conspicuous bundles of myelinated fibres and in a profuse network of very fine unmyelinated fibres This latter is difficult to analyse by micro anatomical methods and the more recent findings have been obtained by a study of the degeneration that follows the operation of prefrontal lobotomy in the human and by the method of physiological neuronography in animals

The following tracts appear at the moment to contribute the main fibre connection of the hypothalamus

1 *Mammillary peduncle* This tract which is conspicuous and well developed in lower mammals is poorly developed in man It has been observed to break away from the medial lemniscus in the midbrain to pass up on either side of the interpeduncular nucleus and terminate in the lateral mammillary nucleus or more rostrally in the hypothalamus The mammillary peduncle may well be an important path for sensory impulses to the hypo thalamus and it has been suggested (Clark 1938) that it bears the same relationship to the hypothalamus that the medial lem niscus does to the thalamus It is felt that further investigation of this tract would be profitable especially since lesions in the mammillary body have been found to abolish the nervous reflex release of A C T H following peripheral stimulation (stress) and since it is probable that afferent nerve impulses passing into the hypothalamus via the midbrain can modify gonadotrophic secretion

2 *Fornix* The evidence indicates that the fornix system con sists of afferent fibres to the hypothalamus It arises from the whole extent of the hippocampus and sweeps around as the columns of the fornix to end in all the nuclei of the mammillary body The function of this tract is unknown The fact that the fornix and hippocampus are highly developed in the human is of interest in relation to the views of Papez (1937) that these struc tures form part of a mechanism underlying emotion Since removal of a temporal lobe including the hippocampus is now being performed in the human for the treatment of certain forms of epilepsy further information regarding the function of the fornix may be expected in the near future

3 *Mammillo thalamic tract (bundle of Vicq d Azyr)* This

Lateral region Through this region runs the medial forebrain bundle which is phylogenetically an ancient system This nerve tract is continuous rostrally into the lateral preoptic area and posteriorly into the tegmentum of the midbrain It connects the more anterior olfactory apparatus with the hypothalamic nuclei since it probably gives off fibres to and receives fibres from these nuclei as it passes caudally At its posterior end it forms one of the main efferent paths from the hypothalamus into the brain stem Scattered among the fibres of the medial forebrain bundle are nerve cells small to medium in size rostrally though larger and more darkly staining posteriorly These cells have been collectively termed the lateral hypothalamic nucleus In the lateral hypothalamic area at the level of the tuber cinereum are groups of small cells termed the tuberal nuclei These nuclei are only prominent in primates in which forms they may project as tubercles on the surface

Supraoptic region This region contains the supraoptic nucleus which straddles the commencement of the optic tract and the paraventricular nucleus which lies immediately beneath the ependyma of the third ventricle These two nuclei are both evolved from the single nucleus preopticus of amphibians and in higher forms are still connected by scattered cells known as the accessory supraoptic nucleus They both form very obvious dense nuclear groups are highly vascular and consist of large spherical cells with eccentric nuclei and peripherally distributed Nissl substance

Tuberal region The dorsomedial hypothalamic nucleus is ill defined The ventromedial hypothalamic nucleus is very clearly seen in the brain of a rodent or human foetus but is less easily seen in the adult human brain Caudal to these two nuclei is a further ill defined zone the posterior hypothalamic nucleus or area which contains mainly small cells amongst which are scattered a few large darkly staining neurones

Mammillary region The mammillary body is composed of several nuclear groups varying somewhat in different forms In the human a medial and lateral mammillary nucleus may be seen (the latter composed of large darkly staining cells) and a nucleus intercalatus

8 *Neocortical connections* It has long been clear on physio logical evidence that connections exist between the cerebral neo cortex and hypothalamus The profuse nature of fibres passing from the hypothalamus to frontal cortex has been summed up by Clark (1948) as follows the greater part of the cortex of the frontal lobe must be regarded as a projection area receiving the products of activity of the hypothalamus in the same way that the visual cortex is the projection area for retinal activities or the auditory cortex for cochlear activities The connections between the mammillary body anterior nuclear complex of the thalamus and gyrus cinguli is described above Another indirect cortical connection via the thalamus lies in the hypothalamic connection with the dorsomedial nucleus of the thalamus through the periventricular fibres This nucleus of the thalamus is in turn connected with the frontal cortex as first described by Clark and Boggon (1933) in lower animals The detailed anatomical studies of Meyer Beck and McLardy (1947) in man and the experi mental findings of Murphy and Gellhorn (1945) also confirm the existence of this path

Fibre connections passing from the frontal cortex to the hypo thalamus have been the subject of much recent study These connections do not pass via the dorsomedial nucleus of the thalamus for a study of human brains after prefrontal lobotomy has shown surprisingly few fibres from the severed areas (pre frontal) terminating in this thalamic nucleus (Meyer 1949) However direct cortical connections to various hypothalamic nuclei have now been established Ward and McCulloch (1947) using the method of physiological neuronography have demon strated connections from cortical area 6a to the mammillary nuclei and posterior and lateral hypothalamic areas from more anterior frontal areas to the supraoptic and paraventricular nuclei and from the medial and posterior orbital cortex to the paraventricular nucleus and posterior hypothalamic areas Micro anatomical methods involving the use of Glee s silver impregna tion method on the brains of patients dying shortly after pre frontal lobotomy have substantiated some of these findings in the human Meyer (1949) has demonstrated a connection pa sing from cortical areas 6 8 and posterior 9 to the mammillary nuclei of the hypothalamus and subthalamus Beck Meyer and leBeau (1951) have described a direct cortico hypothalamic path arising

tract arises mainly from the medial mammillary nucleus and passes dorsally to the anterior nuclear group of the thalamus It is likely however that some of the fibres of the mammillothalamic tract are afferent to the hypothalamus Since the anterior nuclei of the thalamus relay to the gyrus cinguli (Clark and Boggon 1935 Meyer Beck and McLardy 1947) this system forms an important connecting link from the hypothalamus to the neo cortex on the medial surface of the frontal lobe

4 *Mammillo tegmental tract* The mammillo tegmental tract appears to form another efferent path for the medial mammillary nucleus This tract passes into the tegmentum of the midbrain and becomes lost in sections, in a region ventral to the oculo motor nucleus

5 *Medial forebrain bundle* As mentioned above this fibre tract passes from the olfactory areas of the hemisphere caudally through the preoptic region and hypothalamus into the tegmen tum of the midbrain It is probable that fibres both leave and enter, this nerve bundle as it passes posteriorly through the lateral hypothalamic area

6 *Periventricular system* This system of fine fibres lies im mediately beneath the ependyma of the third ventricle It passes from most of the medially situated hypothalamic nuclei vertically upwards to the medial thalamic nuclei (especially the dorso medial thalamic nucleus) So far as hypothalamo thalamic inter relations are concerned, this system forms a two way path The more posterior fibres however, may form an efferent path from the hypothalamus into the midbrain for they sweep ventral to the posterior commissure and pass caudally ventral to the aque duct as the dorsal longitudinal fasciculus of Schutz

7 *Diffuse descending system* The most important descending path from the hypothalamus to the brain stem and spinal cord consists of a diffuse fibre system passing from the lateral hypo thalamic area into the tegmentum of the midbrain Ranson and Magoun (1939) describe these fibres as passing from the hypo thalamic nuclei laterally into the lateral hypothalamic area and thence into the mesencephalic tegmentum in company with the fibres of the medial forebrain bundle These workers suggest that the fibres descend probably with many relays through the tegmentum and reticular formation of the brain stem to the spinal cord

appendage of the brain On these grounds Peremeschko (1867) suggested the term Blutgefassdruse for this structure Later workers noticed a few nerve cells in the glial matrix of the neural lobe but it was not until Cajal (1894) described a tract of nerve fibres passing from a nucleus situated behind the optic chiasma into the pituitary stalk and superior lobe in young mice that the neurohypophysis was recognized as a possibly important structure On histological grounds Cajal suggested that these nerve fibres might be sensory in nature and the idea of a centripetal flow of nerve impulses and later passage of posterior lobe hormones up the pituitary stalk persisted for many years

Before considering the nerve supply of the neurohypophysis in detail it is necessary to emphasize the fact that the anatomical limits of the neurohypophysis include the infundibular process (neural lobe) infundibular stem and median eminence These *three parts which vary in size and shape in different species* together constitute a single gland The grounds on which the median eminence of the tuber cinereum is classified as neurohypophysis rather than hypothalamus are—reactions on vital staining (Wislocki and King 1936) vascular supply (Wislocki 1937 1938 Green and Harris 1947) embryology (Tilney 1936) and cytology (Gersh 1939 Weaver and Bucy 1940) This extension of neurohypophysial tissue up the stalk and into the median eminence is of importance when considering the results of pituitary stalk section In animals with long pituitary stalks (man and monkey) the infundibular stem and median eminence may constitute 12–16 per cent of total gland tissue (Magoun Fisher and Ranson 1939) so that a low stalk section may leave sufficient of the gland still innervated to prevent the onset of signs of hormonal deficiency Fisher Ingram and Ranson (1938) found that lesions of the supraoptico hypophysial tract resulted in atrophy of the median eminence and infundibular stem (as well as the neural lobe) and that after removal of the neural lobe alone the presence of the median eminence and infundibular stem may be sufficient to prevent the onset of diabetes insipidus

A scanty supply of sympathetic fibres derived from the carotid plexus and running with the posterior median hypophysial artery into the neurohypophysis has been described by Dandy (1913) Little is known of these fibres and it may be surmised that they are vasomotor in nature

anterior to the agranular frontal cortex and terminating in the ventromedial and lateral hypothalamic nuclei, though whether this tract arose from the medial orbital or dorsal frontal cortex could not be determined from their material Wall Glees and Fulton (1951) emphasize the importance of the ventromedial hypothalamic nucleus as an end station of orbital fibres in the monkey

The fibre connections between the frontal cortex and hypo thalamus have been considered in a little detail since many of the findings relating to these tracts are of recent origin and since a probable and important correlation may exist between the following facts

(*a*) The profuse hypothalamo thalamo cortical and cortico hypothalamic connections
(*b*) The close functional relationship between the hypothala mus and the neuro and adenohypophysis
(*c*) The pronounced effect exerted by varying emotional states (emotional stress) over the secretion of various anterior and posterior pituitary hormones
(*d*) The recent work of Porter (1954) who investigated the effects of electrical stimulation and the placement of lesions in the orbital and precallosal cingular cortex of the frontal lobe on the secretion of A C T H
(*e*) The marked emotional changes that occur following the operation of prefrontal lobotomy in man and the possi bility of observing endocrine changes following this opera tion

9 *Other hypothalamic connections* Many other hypothalamic connections are described in the literature—such as those passing in the ansa peduncularis stria terminalis stria habenularis supra optic commissures and other nerve tracts and those suggested by experimental observations such as vago supraoptic connec tions

Nerve supply to the neurohypophysis

Although the nervous nature of the neurohypophysis was recognized by Luschka in 1860 who proposed the name Ner vendruse the general belief was held during the latter half of the nineteenth century that the gland was merely a neuroglial

Kary (1924) and Lewy (1924) first described such degeneration The number of cells in each supraoptic nucleus has been estimated at about 60 000 (man) 30 000–40 000 (monkey) 35 000–40 000 (dog) and 7 000 (rat) (Magoun and Ranson 1939 Rasmussen 1940 Rasmussen and Gardner 1940 Merrick 1944) Following damage to the supraopticohypophysial tract 70–90 per cent of these cells atrophy and disappear (about 70 per cent if the tract is cut at the level of the diaphragma sellae and about 90 per cent if the tract is cut above the median eminence) A similar disappearance of cells occurs in the paraventricular nucleus In the dog about 90 per cent of paraventricular cells disappear after sectioning the supraopticohypophysial tract above the median eminence (Pickford and Ritchie 1945, O Connor, 1947) In view of these findings the recent claim that regeneration of the neurohypophysis occurs after hypophysectomy must be regarded as of very doubtful validity The origin of the supraopticohypophysial tract from the supraoptic and paraventricular nuclei is of special interest when it is remembered that these two nuclei are represented in lower vertebrates by one common nucleus the nucleus preopticus (Meyer, 1935) Other hypothalamic nuclei which have been suggested on histological grounds as giving rise to some fibres of the supraopticohypophysial tract are the nucleus hypothalamicus ventralis pars centralis (Young 1936) anterior hypothalamic ventromedial hypothalamic and ventral periventricular hypothalamic nuclei (Fisher Ingram Hare and Ranson 1935 Fisher, Ingram and Ranson 1935 Green 1951) These sites of origin are however difficult to substantiate since the fibres in question are very fine and hard to trace in serial sections A similar observation applies to the origin of the tuberohypophysial tract which has been given as the central and posterior parts of the hypothalamus possibly from the nucleus periventricularis ventralis scattered cells and nuclei in the tuberal region and mammillary bodies (Ranson Fisher and Ingram 1938 Vidal 1942 Green 1951)

As the supraopticohypophysial tract enters the median eminence it occupies a very superficial position where it is liable to be affected by basal infections of the brain In this region the fibres have been described as pursuing a sinuous course and of undergoing partial decussation However since hemisection of the pituitary stalk leads to appreciable retrograde degeneration in the

The main nerve supply, both functionally and anatomically, of the neurohypophysis is the hypothalamo hypophysial tract in the pituitary stalk Since the time of Cajal this tract has been the subject of many studies A comprehensive list of references (up to 1938) to work dealing with the anatomy of the hypothalamo hypophysial tract in all types of vertebrates may be found in the monograph of Fisher, Ingram and Ranson (1938)

The hypothalamo hypophysial tract consists of two main parts the supraopticohypophysial tract running in the anterior or ventral wall of the stalk and the tuberohypophysial tract in the posterior or dorsal wall of the stalk More information is available concerning the origin function and termination of the former than of the latter component In lower forms fishes and cyclostomes a well marked lateral division of the tract converges to the pituitary stalk through the lateral region of the tuber cinereum (Green 1951)

The nuclei of origin of the fibres in the hypothalamo hypophysial tract have been investigated by two main methods by micro anatomical study of the fibre tracts and by studies of the *retrograde degeneration that occurs in some hypothalamic nuclei* following lesions in the tuber cinereum and infundibular stem Microscopic examination shows that the origin of the greater part of the supraopticohypophysial tract is from the supraoptic *and paraventricular nuclei of the hypothalamus Pines (1925)* first traced the source of some of these fibres as the supraoptic nucleus (nucleus hypophyseus of Pines) This finding was confirmed by Greving (1926) who also described fibres arising in *the paraventricular nucleus running either to the supraoptic* nucleus or to join the fibres in the supraopticohypophysial tract Laruelle (1934) further substantiated the origin of fibres from the paraventricular nuclei These findings have been confirmed by *many workers and more recently have been investigated in* detail by use of the Gomori stain This method (see Chapter 11) differentially stains the neurohypophysis the nerve fibres in the supraopticohypophysial tract and their nuclei of origin Clear histological *pictures of this innervation are thereby* obtained and the findings are in agreement with the older data Further confirmation has been forthcoming from study of the retrograde degeneration of cells in the supraoptic and paraventricular nuclei which *occurs after section of the* supraopticohypophysial tract

The pars intermedia is the only division of the gland that is poorly vascularized compared with other tissues of the body

The neurohypophysis is vascularized from two sources Firstly the median eminence receives a blood supply via the primary plexus of the hypophysial portal vessels This is derived (as described in Chapter 2) from the rich plexus of the pars tuberalis which is in turn supplied with blood through small arterial twigs from the internal carotid artery or circle of Willis Secondly the neural lobe or infundibular process possesses an arterial supply and venous drainage which is separate from that of other parts of the gland The details of this supply vary in different forms In the rabbit the neural lobe is usually supplied by a small twig from the right internal carotid artery which takes origin as the parent vessel pierces the dura near the anterior clinoid process This small artery passes posteriorly on the diaphragma sellae sweeps across the upper surface of the neural lobe in the form of an arc from which small branches pass vertically down to supply the infundibular process In the dog a branch passes from the internal carotid artery as it lies in the cavernous sinus medially to anastomose with its fellow in the midline at the posterior pole of the pituitary (Dandy and Goetsch 1911) From this anastomosis posterior median arteries pass forward directly into the neural lobe Jewell (1951) has confirmed this supply and has noticed that the parent branches from the carotid may be asymmetric In the human the inferior hypophysial artery from the cavernous part of the internal carotid divides into a medial and lateral branch (McConnell 1953 Xuereb Prichard and Daniel 1954) These branches anastomose with corresponding vessels of the opposite side forming an arterial ring around the junction of the neural lobe and pars distalis It is from this ring that the infundibular process is supplied

The blood supply of the third part of the neurohypophysis the infundibular stem varies In some cases as in the human this is by means of an extension of the primary plexus of the portal vessels from the median eminence into the infundibular stem In other forms such as the rabbit the infundibular stem appears to be vascularized in common with the neural lobe In still other animals such as the dog the infundibular stem hardly exists For this reason Green (1951) has suggested that the neurohypophysis should be subdivided into two regions only the

supraoptic nucleus of the same side only such decussation cannot be of any magnitude The tuberohypophysial tract in the dorso caudal region of the median eminence is smaller and consists of finer fibres In the neural stalk all the fibres congregate into a dense bundle lying in a central position leaving a peripheral zone in contact with the pars tuberalis relatively free of nerve elements The number of fibres in this site has been estimated at about *10 000 in the rat about 60 000 in the dog and monkey and about* 100 000 in man (Rasmussen 1940)

The termination of the fibres of the hypothalamo hypophysial tract is mainly in the neurohypophysis A few fibres have been seen in many forms passing into the pars tuberalis and pars inter media of the adenohypophysis but there is no good evidence for the belief that they penetrate the pars distalis It is probable that the tract terminates in all three parts of the neurohypophysis since as mentioned above more cells in the supraoptic and para ventricular nuclei degenerate following lesions above or in the *median eminence than in the hypophysial stalk* The histo logical endings of the nerve fibres in the neural lobe are obscure Although a variety of nerve terminals have been described such as terminal enlargements bulbs menisci varicose extremities and so on there is no common agreement as to secretory cells in the neurohypophysis or as to secreto motor endings It may be significant in this respect that many authors agree that peri vascular arborizations and endings are common in the *neuro* hypophysis (see Vazquez Lopez 1942 and Green 1951) Such types of endings are very conspicuous in certain forms such as the opossum (Green 1951 Bodian 1951) and are of interest in view of the possibility that the mechanism of the secretion of posterior pituitary hormones may be direct liberation of the hor mone from nerve terminals into blood vessels

The blood supply of the neurohypophysis

Contrary to the statements found in many textbooks the neurohypophysis is very richly vascularized Judging from the histological appearance of specimens in which the vascular sys tem has been perfused with some colouring material the pars tuberalis is the most highly vascularized part of the pituitary with the pars distalis and neural lobe following in that order

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median eminence and the neural lobe These he defines as follows—the median eminence is that part of the neurohypophysis which receives its blood supply from the hypophysio portal circulation or (in order to include the agnatha and fishes) which has a common vascularization with the adenohypophysis—and the neural lobe, the remainder of the neurohypophysis independently supplied with blood The term infundibular stem he regards as having no exact meaning but being of descriptive value only Green has also proposed the interesting view that the median eminence and infundibular stem are evolved separately He described the median eminence as being already present in cyclostomes and fishes, in which a common circulation exists between this structure and the adenohypophysis (analogous to the hypophysial portal circulation of higher forms) However it was not until amphibians and reptiles were reached that is forms which are becoming or have become independent of an aquatic environment that a true neural lobe appeared This new structure retains in higher forms the hall mark of its separate origin in the form of independent vascularization

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CHAPTER 9

REGULATION OF THE SECRETION OF ANTIDIURETIC HORMONE

In the eighteen years between 1895 and 1913 the main pharmacological activities of posterior pituitary extract were discovered It was found that injection of such extracts had a pressor action (Oliver and Schafer 1895 Howell 1898) a diuretic action in *anaesthetized* animals (Magnus and Schafer 1901 Schafer and Herring 1906) an antidiuretic and chloruretic action in *unanaesthetized* animals (von den Velden 1913) a hyperglycaemic action (Borchardt 1908) a stimulating action on intestinal peristalsis (Bell 1909) an oxytocic action (Dale 1909) a galactogogue action (Ott and Scott 1910) and other effects Since these pioneer studies the above and other actions of posterior lobe extracts have been investigated in detail In 1928 the single crude extract with multiple activities was purified and separated into two fractions by Kamm Aldrich Grote Rowe and Bugbee working in the research laboratories of Parke Davis & Co The names and probable activities of these fractions are shown below

		Probable activities
Whole posterior pituitary extract (Pituitrin Parke Davis & Co)	Pressor fraction Vasopressor fraction Antidiuretic fraction (Pitressin Parke Davis & Co)	Pressor Antidiuretic Chloruretic Stimulation of intestinal peristalsis Hyperglycaemic
	Oxytocic fraction (Pitocin Parke Davis & Co)	Oxytocic Galactogogue

More recently du Vigneaud and his colleagues (du Vigneaud Ressler Swan Roberts Katsoyannis and Gordon 1953 du Vigneaud Lawler and Popenoe 1953) have succeeded in obtaining these fractions in pure forms It should be emphasized that the problem of many years standing as to whether the neurohypophysis secretes one hormone or two is not yet settled In the following discussion antidiuretic hormone and oxytocic hormone will be referred to separately according to current usage

but this should not be taken to imply that they are separate hormones The evidence on this point is inconclusive

Data regarding regulation of the secretion of the antidiuretic hormone has been obtained from three main lines of investiga tion Studies have been made of (*a*) lesions of the supraoptico hypophysial tract in patients and animals (*b*) direct electrical stimulation of the gland or its nerve supply, and (*c*) changing the external or internal environment in such a way that the secretion of the gland is increased (indirect stimulation)

(*a*) Lesions of the supraopticohypophysial tract

As has been discussed by Fisher Ingram and Ranson (1938) the history of our knowledge concerning the aetiology of dia betes insipidus is a chapter of errors errors not of observation but of the conclusions drawn Magnus and Schafer (1901) and Schafer and Herring (1906) first showed that extracts of the pos terior pituitary have a diuretic effect on injection into anaesthet ized animals and on the basis of this observation it was thought that lesions of the pituitary which result in diabetes insipidus do so as a result of irritation However in 1913 von den Velden and Farmi independently showed that posterior pituitary extract exerts an antidiuretic effect on injection into clinical cases of diabetes insipidus and it later became clear that a diuretic effect was dependent on the anaesthetized state The view was then held that diabetes insipidus was due to deficiency of posterior lobe secretion due to damage to the pituitary In 1920 however Camus and Roussy showed that damage to the hypothalamus in the presence of an intact pituitary could result in this condition Thus evolved two divergent views that diabetes was of hypo thalamic origin or of pituitary origin This controversy was slowly settled by the redescription by Pines (1925) and Greving (1926) of the rich nerve tract first seen by Cajal in 1894 that unites the hypothalamus and hypophysis It then became apparent that the hypothalamus and posterior pituitary function as a unit and that damage to either may result in polyuria This view did not find universal acceptance for some years since it was known that complete hypophysectomy does not necessarily result in diabetes insipidus and since interruption of the pituitary stalk in some forms such as the monkey (Mahoney and Sheehan 1936) is compatible with a normal urine volume These two

difficulties were finally reconciled with the general view of a hypothalamo hypophysial unit through the work of von Hann (1918) and Richter (1934) who showed that the anterior lobe of the pituitary exerts a diuretic effect without which the result of posterior pituitary deficiency on urine output may not be apparent (see however Heinbecker White and Rolf 1947) and through the finding that the median eminence and the pituitary stalk contain neurohypophysial glandular tissue so that section of the stalk in some forms does not completely denervate the whole neurohypophysis

The clarification of the picture relating hypothalamic and hypophysial lesions to diabetes insipidus is due to the work of Ranson and his colleagues The results obtained by this group is presented with a full discussion in the monograph by Fisher Ingram and Ranson (1938) The technical side of this work was much simplified by the use of the Horsley Clarke stereotaxic instrument This is a precision instrument originally designed and used by Sir Victor Horsley and Dr R H Clarke (1908) for the accurate placement of small lesions in different parts of the cerebellum By the use of this instrument Fisher et al made localized electrolytic lesions in various parts of the hypothalamus of cats They noticed that bilateral lesions which interrupted the supraopticohypophysial tract resulted in a condition similar to that of clinical diabetes insipidus This was observed in 64 animals in which the daily urine output was markedly raised above the normal (normal output for the cat is about 100 ml / day) The onset of diabetes in the majority of cats occurred in the following three phases post operative days 1–6 phase of transient polyuria urine output up to 500 ml /day days 6–10 normal interphase normal urine output days 10 and onwards phase of permanent polyuria output 300–500 ml /day Similar phases have been observed during the onset of diabetes insipidus in man (Gagel and Klaes 1950) It is likely that the normal interphase is a period when the denervated neurohypophysis is under going atrophy and that simple reabsorption of the hormonal con tent of the gland is sufficient to maintain a normal urine volume over this period O Connor (1952) reported that a normal inter phase does not occur if the posterior pituitary of the dog is removed after cutting the stalk though this phase is present fol lowing simple stalk section During the phase of permanent

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polyuria, Fisher et al found that the urine output could be brought down to normal levels by replacement therapy with pos terior pituitary extracts After death histological examination of the hypothalamus pituitary region of the animals that had had diabetes showed, firstly that the supraopticohypophysial tracts had been interrupted bilaterally, secondly disappearance of the greater number of nerve fibres in the neurohypophysis and atrophy and loss of nerve cells in the supraoptic nuclei and thirdly an atrophy and hypercellularity in all parts of the neuro hypophysis The various parts of the adenohypophysis of these cases appeared normal though the pars intermedia was wrinkled over the shrunken neural lobe In 39 control cats in which lesions had been placed in other parts of the hypothalamus and in which diabetes had not occurred similar atrophic changes were not found Extracts of the atrophic neurohypophyses associated with the diabetic state were found to possess greatly diminished pressor antidiuretic and oxytocic activity though the melanophore expanding activity of these pituitary glands was normal These results in the cat were confirmed in the monkey, both by placing electrolytic lesions in the hypothalamus and later by Magoun Fisher and Ranson (1939) by exposing the pituitary stalk in this animal by a subtemporal approach and making cuts in the median eminence or infundibular stem Whereas complete transection of the median eminence or of its anterior half resulted in marked polyuria transection of the in fundibular stem, leaving the median eminence part of the neuro hypophysis still normally innervated produced a less severe polyuria if any at all These results are enlightening with regard to the results of Mahoney and Sheehan (1936) on the monkey and Dandy (1940) on the human who found no or only mild polyuria following section of the pituitary stalk Magoun et al (1939) found the median eminence normally constitutes 13 per cent of total neurohypophysial tissue in the monkey They com ment that the maintenance of a normal urine output by 1/6th to 1/8th of the normal amount of neurohypophysial tissue indicates a physiological reserve comparable with that of other endocrine organs

The magnitude of the polyuria observed in diabetic animals belonging to different species varies Average figures for the daily urine volume under these conditions are about 100–200

c c /kg body weight for the cat monkey and man about 300 c c /kg for the dog and often 1 000 c c /kg for the rat Only a transient primary polyuria but no permanent polyuria has been observed to follow the placement of lesions causing complete atrophy of the neurohypophysis in rabbits (Cross and Harris 1952) It is interesting to compare these figures with those for the amount of glomerular filtrate formed per day Cushny (1926) calculated that the cat forms 12 litres of glomerular filtrate per 24 hours Since the urine volume of the cat is approximately 100 c c /24 hours it follows that the tubules of the cat kidney re absorb 11 9 litres/24 hours Under conditions of diabetes in sipidus in which the effect of the antidiuretic hormone on the kidney tubules is lacking the urine volume may rise to about 1 000 c c /day which indicates a tubular reabsorption in the order of 11 0 litres/24 hours As pointed out by Fisher et al (1938) since the antidiuretic hormone is only responsible for 1 litre of the 11 9 litres reabsorbed by the tubules per day other factors must play an important part in determining the level of such polyuria In support of this view they showed that depriving diabetic cats of fluid would reduce the urinary output to normal levels Similarly the fact that rabbits do not show a permanent polyuria is probably due to the intervention of some other factor possibly a change in renal haemo dynamics

The overall picture regarding the supraopticohypophysial system and the effects of lesions in this system as described by Ranson and co workers has changed little Further work has firmly substantiated the views of the Chicago group in spite of a few publications that have suggested that the neurohypophysis is a sensory chemoreceptor organ The basis of this latter hypothesis was histological study of the gland and on the available data does not merit serious attention

More detailed studies have been made in recent years (White Heinbecker and Rolf 1942 Pickford and Ritchie 1945 and Heinbecker White and Rolf 1947) regarding the part played by the anterior lobe of the pituitary in the development of a maximum diabetes insipidus As mentioned above animals deprived of the pars distalis as well as the neurohypophysis develop only a slight polyuria if any at all Besides the milder diabetes such animals show a reduced diuretic response to ingested water and reduced insulin creatinine and urea clearances These effects do

not appear to be due to loss of the thyrotrophic hormone and are not repaired by administration of suprarenal cortical extracts or desoxycorticosterone acetate It is unlikely that the anterior pituitary secretes a specific diuretic hormone and the evidence so far suggests that the effects observed in the absence of this gland are due to changes in the vascular condition of the kidney (White et al, 1942)

(*b*) Electrical stimulation of the supraopticohypophysial tract and neurohypophysis

If the views of Ranson are correct that the supraopticohypophysial nerve tract regulates the secretion of the antidiuretic hormone it would be expected that electrical stimulation of this tract would elicit signs of A D H secretion This obvious experimental corollary to the work of Ranson and his group was not however forthcoming for several years since it is necessary to study liberation of the antidiuretic hormone against a background of water diuresis and since surgical anaesthesia prevents the occurrence of a water diuresis

Haterius (1940) investigated the effects of several types of anaesthesia on water diuresis and found that a mixture of chloralose and urethane injected intravenously had the least depressing action on the excretion of water Using this form of narcosis in rabbits he clamped and orientated the animals heads in a stereotaxic instrument and inserted electrodes into the region of the pituitary stalk This manipulation invariably caused abrupt diminution in the rate of urine flow and further administration of water by stomach tube frequently resulted in death However good diureses were eventually obtained in 8 experimental and 6 control animals Of the 8 experimental animals 5 showed a definite antidiuresis on stimulation of the pituitary stalk and 3 only transient depression In one of these 3 the electrodes were found at post mortem to be 1–2 mm lateral to the stalk In the 6 control rabbits that had had electrolytic lesions placed in the region of the stalk two to four days previously 4 failed to show an antidiuresis on stimulation In the 2 animals in which stimulation checked the diuresis autopsy revealed intact pituitary stalks

The problem was further investigated (Harris 1947) after the remote control method of stimulation for the basal areas of the

brain had been developed This technique allows repeated stimulation to be performed in the conscious animal and therefore gives freedom from the complications of anaesthesia Stimulation in these experiments was performed by holding a primary coil a fixed distance from the surface of the scalp over the embedded secondary coil (after measuring the thickness of the soft tissues of the scalp radiographically) and was applied to a variety of regions of the hypothalamus and pituitary gland Over 300 experiments on 27 rabbits gave the following results If the electrode tip was in contact with the supraopticohypophysial tract in the hypothalamus median eminence infundibular stem or process stimulation was found to produce a temporary inhibition of a water diuresis in the hydrated animal The duration of the inhibition varied from 15 minutes to several hours according to the intensity of the stimulus In any one animal repeated experiments showed that the response to a given stimulus remained remarkably constant over periods of weeks or months Both the antidiuresis and the increase of chloride concentration of the urine which followed stimulation could be matched by intravenous injection of appropriate doses of whole posterior pituitary extract (Pituitrin Parke Davis & Co) or the purified pressor fraction (Pitressin Parke Davis & Co) In animals in which the electrode tip was situated within ½ mm of the supraopticohypophysial tract antidiuretic responses followed stimulation but only if the stimulus was strong In cases where the electrode was further from the S O H tract than ½ mm i e in various regions of the hypothalamus thalamus or pars distalis of the pituitary the strongest stimulus it was possible to administer to the conscious animal failed to elicit a response It could therefore be said that the spread of stimulus effective in exciting the fine unmyelinated fibres of the supraopticohypophysial tract was not more than ½ mm

There can be little doubt that the antidiuretic and chloruretic effects produced by electrical stimulation of the supraoptico hypophysial tract are due to release of the antidiuretic hormone It was possible to compare the smaller responses with those produced by injection of small doses (1–5 mU) of posterior pituitary extract However the larger responses that followed more intense stimulation were found to be outside the range of accurate assay but from various lines of evidence it is believed they were

the result of the liberation of antidiuretic hormone corresponding to about 50 mU of posterior pituitary extract It is interesting to compare these figures with the findings that the neurohypophysis of the dog normally liberates antidiuretic hormone at a rate of 1 0–5 0 mU/hr (Shannon, 1942) or about 3 6 mU/hr (Verney 1946) and that an emotional stress stimulus in this species may result in the release of 5–10 mU of hormone (O Connor and Verney, 1942 O Connor, 1946)

The results of experiments involving lesions or stimulation of the supraopticohypophysial tract are in good general agreement

(*c*) Humoral and nervous reflex excitation of the supra opticohypophysial system

The above discussion has been focussed on the supraoptico hypophysial tract as the nerve tract responsible for regulating the secretion of the antidiuretic hormone and from the above and other data it seems clear that the neurohypophysis is as fully dependent on its nerve supply for activity as is a voluntary muscle In the absence of this nerve supply the gland undergoes atrophy and function ceases

The factors which are at present known to influence the activity of the supraopticohypophysial system and the secretion of A D H are summarized in Fig 49 The more constant factor regulating A D H secretion appears to be the osmotic pressure of the blood In one sense this forms a type of feedback mechan ism affording reciprocity between the activity of the kidney (and intestines lungs and sweat glands) and the neurohypophysis Superimposed upon this more basic mechanism is that due to nervous reflexes including those excited by emotional stresses which probably act through a variety of nervous pathways ending on the supraoptic nuclei

The broad outline of the mechanisms regulating the activity of the neurohypophysis (as depicted in Fig 49) are very similar to those concerned with the activity of the adenohypophysis (as depicted in Figs 31 and 42) Firstly the activity of the gland is largely or completely dependent on its connections with the hypothalamus Secondly the resting activity of the gland under optimum quiescent conditions is stabilized by a feedback mechanism, related to changes in the internal environment and to some peripheral effect produced by the hypophysial hormone

(kidney excretion and osmotic pressure of the blood or blood level of target organ hormone) Thirdly superimposed on the above is the effect of the central nervous system which relates hypophysial activity to changes in the external environment The effects produced by changes in the external environment seem to be closely associated with states of emotional stress and appear to act by nervous reflex paths involving the hypothalamus

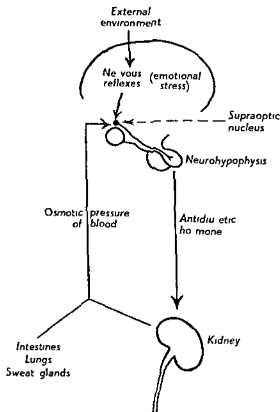


Fig 49 To illustrate the ma n f ctors affecting the secretion of ant diuretic hormone The base l ne level of secretion is determined by the osmotic pressure of the blood to wh ch some reg on in the fo ebrain (probably the supr optic nucleus) is sens t e Stimuli arising in the external en ironment ffect the neurohypophysis either by nervous reflex paths or ndirectly by in ducing a state of emot onal stress These latter stimuli take precedence o er the forme as may be seen by the inhibition of a vater d u esis that follo s a pa nful st mulus

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Verney's theory that hydration of an animal results in inhibition of posterior pituitary secretion of antidiuretic hormone was compatible with findings soon forthcoming that dehydration resulted in signs of neurohypophysial activation Simon (1934) and Simon and Kardos (1934) found that the neurohypophysis of rabbits guinea pigs and cats kept for 4–5 days on dry diet was largely depleted of its hormone content Gilman and Goodman (1937) discovered an antidiuretic substance in the urine of rats dehydrated by deprivation of water or administration of hypertonic NaCl This substance had properties similar to that of posterior pituitary extracts and was not found in the urine of normal animals or of dehydrated hypophysectomized animals These observations have been confirmed by many workers though the posterior pituitary origin of the urinary antidiuretic substance has been questioned However the fact that direct electrical stimulation of the supraopticohypophysial tract in the normal rabbit causes the appearance of an antidiuretic substance in the urine (Harris 1948) lends support to the view that the substance observed after dehydration is of pituitary origin

There is now much evidence that administration of hypertonic saline causes discharge of the antidiuretic hormone for such a procedure will inhibit a water diuresis (Hare Hare and Phillips 1943 Chambers Melville Hare and Hare 1945) and will produce histological changes (Chambers 1945*a*) and loss of antidiuretic hormone (Chambers 1945*b*) in the neurohypophysis of the rat

The mechanism whereby injection of hypertonic solutions result in activation of the neurohypophysis has been studied in detail by Verney who has published reviews of his findings (Verney 1946 1947) In this work Verney used bitches that had been perineotomized in order to facilitate catheterization of the bladder and in which carotid loops' had been prepared thus enabling the injection or perfusion of various solutions to be made into the common carotid artery He first established that injection of isotonic solution of sodium chloride (up to 20 c c 0 144 M NaCl in 20 sec) into the carotid artery or injections of hypertonic solutions (up to 20 c c 0 343 M NaCl in 20 sec) intravenously did not inhibit a water diuresis However injections of hypertonic solutions of sodium chloride (such as 20 c c of 0 343 M NaCl in 25 sec) into the carotid artery produced

Fourthly in both cases the stimulating effect of stress predominates over an inhibitory influence derived from the internal environment (for example lowered osmotic pressure of the blood as during a water diuresis or increased blood level of adrenal cortical hormones) Fifthly the activity of both the adenohypophysis and neurohypophysis appears to be affected in some way by the secretion of adrenaline

Apart from the diuresis that occurs during a state of diabetes insipidus there are two other conditions characterized by a similar profuse watery diuresis i e the isolated kidney and changes in osmotic pressure of the blood

1 *Isolated kidney* Verney and Starling (1922) and Starling and Verney (1925) showed that perfusion of a dog s kidney in the isolated state results in the formation of urine with characters similar to that seen in diabetes insipidus that is of high volume low specific gravity and low chloride concentration On addition of posterior pituitary extract to the blood circulating in the heart lung kidney preparation the volume of urine formed was reduced and the chloride concentration increased Similar effects were noted by Verney (1926) if a perfused head neck preparation was switched into the heart lung kidney circuit though not if the pituitary gland had been previously removed from the head It was then clear that the kidney isolated from the antidiuretic secretion of the pituitary secretes large volumes of urine but of low concentration

2 *Osmotic pressure of the blood* Another condition in which a similar diuresis is seen is following ingestion of water by mouth In a study of this state Klisiecki Pickford Rothschild and Verney (1933*a*) administered water to bitches with exteriorized ureters and noted a lag period of 15 minutes between the peak water load of the tissues and the peak of the diuresis curve It was found that the diuretic response of the denervated kidney runs parallel with that of the innervated kidney and also (Cowan Verney and Vogt 1938) that a water diuresis is unaccompanied by any change in blood flow through the kidney The suggestion was put forward (Klisiecki et al 1933*a* and *b*) that ingestion and absorption of water results in a decreased osmotic pressure of the blood inhibition of the secretion of antidiuretic hormone with a consequent diuresis which begins after the hormone already in the blood stream has been removed or inactivated

chloride was acting specifically in eliciting the response to intra carotid injections or by virtue of the increase it produced in the osmotic pressure of the plasma comparison was made of the effects of approximately isosmotic increases produced by solutions of sodium chloride glucose sucrose sodium sulphate and urea It was found that for equal increments in the osmotic pressure of the carotid blood produced by sodium chloride sucrose or sodium sulphate equal amounts of antidiuretic hormone were liberated In this respect glucose was slightly less effective and urea was in effective In view of the fact that the only common and equal change in property of the carotid blood caused by the injection of for example the solutions of sodium chloride and sucrose was the increase in osmotic pressure and that both solutions pro duced quantitatively the same release of posterior pituitary anti diuretic substance the conclusion was drawn that the effect was osmotically determined From this it followed that some recep tive elements are present in the field of the carotid supply which are sensitive to changes in the osmotic pressure of this arterial blood

The site of the osmoreceptors is unknown Verney showed that the carotid body and the carotid sinus are not the site for denervation of these structures did not affect the responses ob tained The field of search has been further narrowed by the following observations (*a*) ligation of the internal carotid artery abolishes the response to the injection of hypertonic solution of sodium chloride in the corresponding common carotid artery (Verney 1947) (*b*) equal responses followed injection into both common carotid arteries of a dog in which the neural lobe of the pituitary received an asymmetric arterial supply (Jewell and Ver ney 1953) and (*c*) the results of intracarotid infusion of hyper tonic solutions in an animal before and after intradural ligation of one internal carotid suggests the osmoreceptors lie in some part of the prosencephalon (Jewell and Verney 1953) (*d*) section of the pituitary stalk abolishes the antidiuretic response to injection of hypertonic saline but if the diencephalon is isolated from peripheral receptors by cutting the midbrain the cervical sym pathetic trunks and the first three cranial nerves the response is still retained (Hare 1947) The extremely vascular nature of the supraoptic nuclei would suggest these cell groups are strong can didates for the site of the osmoreceptors Verney (1947) noticed

marked inhibitory responses in the course of a watery diuresis (Fig 50) responses that very closely simulated those following intravenous injection of posterior pituitary extract The pituitary origin of the antidiuretic response elicited by intracarotid injec

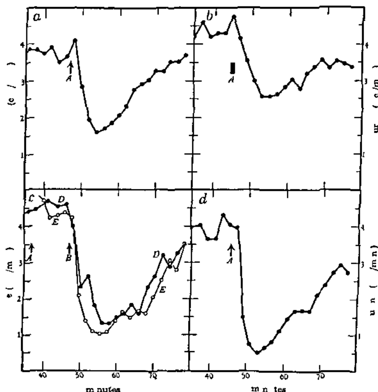


Fig 50 Antidiuretic responses produced by injection of hypertonic NaCl into the carotid artery

a At A injection of 20 c c 0 57 M NaCl into left carotid in 16 sec *b* At A injection of 12 c c 0 343 M NaCl into left carotid in 60 sec *c* At A injection of 12 c c 0 139 M NaCl into left carotid in 10 sec and at B injection of 12 c c 0 343 M NaCl into right carotid in 15 sec (graph D) and in 10 sec (graph E) *d* At A injection of 20 c c 0 343 M NaCl into left carotid in 25 sec Abscissae time after the test dose of water

(From Verney E B (1947) *Proc Roy Soc B* 135 25)

tion of hypertonic solutions of sodium chloride was confirmed by the observation that surgical removal of the neural lobe of the pituitary reduced the response to about 10 per cent of that obtained previously In order to find out whether the sodium

also result in a decreased urine flow and suggested this might be due to the emotional accompaniment of the procedure [1] The first detailed analysis of this phenomenon was made by Rydin and Verney (1938) who investigated the mechanism involved in the antidiuresis produced in dogs by forced running on a treadmill

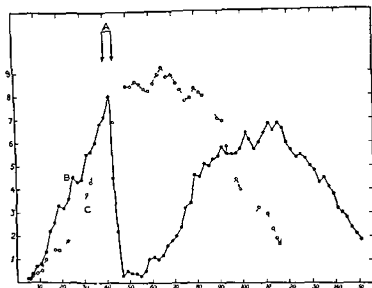


Fig 51 Antidiuretic response produced by exercise
Water diuresis curves from a fox terrier bitch R V 4 Fundus of bladder excised and urethral cannula implanted 10 days before 250 c c water given at zero time B = response on 7th August 1935 C = response on 8th August 1935 The response on August 6th was closely parallel with C A = period of exercise running at 6 m p h during response B Ordinate = rate of urine flow in c c /2 min Abscissa = time in minutes
(From Rydin H & Verney E B (1938) *Quart J exp Physiol* **27** 343)

They found that mild exercise—for example running at 4–5 m p h for 4 minutes—was generally followed by a marked and prolonged inhibition of urine flow (Fig 51) For the following reasons however they were led to the conclusion that the anti diuretic effect of exercise was not due to the exercise *per se* but

[1] This observation calls to mind the finding that the unexercised coach or coxswain as well as the rowing crew involved in a boat race are stimulated to secrete A C T H from the anterior pituitary with a resultant eosinopenia (Renold Quigley Kennard & Thorn 1952)

small vesicles situated in the supraoptic nuclei of the dog and as he says considered the ascription of an osmotic function to these structures as a speculative indulgence Jewell (1953) studied the cytological features of these vesicles further He found that they are intracellular structures in close proximity to, but not connected with the capillary plexus of the nucleus His observations were compatible with the view that they function as minute osmometers

More prolonged experiments involving intracarotid infusions as opposed to injections of hypertonic solutions have been performed (Verney 1947) in an attempt to gather information regarding the smaller and more prolonged changes that occur in the osmotic pressure of the blood in the normal animal In this connection 10 and 40 minute infusions showed that osmotic changes in the carotid blood when reduced to a range well within that which may reasonably be regarded as physiological are still operative in causing the release of post pituitary antidiuretic substance It is likely from these results in the dog that an increase of only 1 per cent in the osmotic pressure of *aortic* blood would reduce a water diuresis to only 10 per cent of the maximum rate of urine output which corresponds to a release of about 1 μU/sec of antidiuretic substance Such changes in osmotic pressure are within the range of falls reported in water diuresis in man A further finding of interest in regard to various clinical states is that the osmoreceptors adapt to infusions of glucose and urea The process of adaptation probably reflects a permeability of the membrane of the osmoreceptors to these substances and explains why patients with a high level of blood glucose for example those suffering from diabetes mellitus can be polyuric

Emotional stress

Claude Bernard reported in 1859 that the rate of urine secretion was reduced during states of emotional stress associated with with operative procedures in the human This phenomenon was observed during operations by Jobert on certain cases of vesico vaginal fistulae It has also been known for many years that muscular exercise is related to inhibition of a water diuresis MacKeith Pembrey Spurrell Warner and Westlake (1923) who studied the effect of exercise noted that a feigned run could

response to emotional stress is due to some agent humorally conducted to the kidney and that this agent is not adrenaline Since the course of the inhibitory response could be matched with very small doses of post pituitary extract it seemed likely that it was due to reflex excitation of the neurohypophysis This view has been put to the experimental test by O Connor and Verney (1942) who studied the effect of emotional stress on a water diuresis after removal of the posterior lobe of the pituitary and by O Connor (1946) who made similar studies after section of the supraopticohypophysial tract Both operative procedures resulted in greatly reduced antidiuretic responses responses that assayed at only about 5 per cent of those present before opera tion There can be little doubt then that sensory stimuli of various types which result in the so called state of emotional stress will activate nervous pathways to the supraoptic nuclei which in turn excites the release of antidiuretic hormone from the neurohypophysis

The effect of sensory nerve stimulation on the excretion of water by the kidney has been frequently attributed to activation of the neurohypophysis For example this explanation has been put forward with more or less data in its support to explain the renal effects observed after retinal stimulation with flashing light in the rat (Boyd Lee and Stevens 1943) electrical stimulation of the vagus nerve in the dog (Chang Chia Huang and Lim 1939) and sensory stimulation of the lumbar area (Theobald and Verney 1935 Haterius 1940) It might be argued that in some of these cases the position is really akin to that associated with exercise that it is not the procedure itself which is ultimately responsible for the excitation of the neurohypophysis but rather the associated emotional stress For example Theobald (1934) found in the dog that not only lumbar puncture but also shaving the skin of the back or even ostentatious preparations for the operation might result in an antidiuretic response In the human Noble and Taylor (1953) have confirmed the release of anti diuretic hormone in relationship to fainting and comment that this is to be related to the fainting and only indirectly to the stimulus which initiates the faint The practical implications of this argument are firstly there would be no good grounds for expecting direct nervous connections between the ascending sensory tracts in the midbrain and the supraoptic nuclei and,

the emotional stress accompanying the exercise Firstly if the animals were repeatedly exercised the inhibitory response progressively diminished to final extinction Secondly, by combining with exercise (the antidiuretic effect of which had been extinguished by repetition) an unpleasant frightening noise such as the sounding of a car horn it was possible to produce an inhibitory response And thirdly emotional stress alone either due to

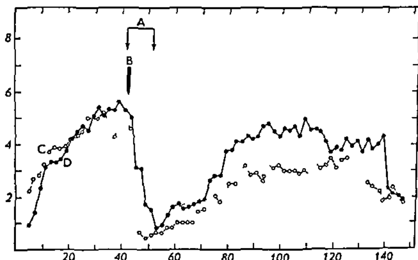


Fig 52 Antidiuretic response of a mild pain stimulus compared with injection of posterior pituitary extract

Water diuresis curves from bitch R V 11 250 c c water given at zero time A = *period of annoyance with mild faradic stimulation while curve D was being* obtained five stimuli were given each of one second s duration B = time of intravenous injection of 1 c c 0 85 per cent NaCl containing 10 ^{4} c c post pituitary extract this was injected while curve C was being obtained Ordinate = rate of urine flow in c c 2 min Abscissa = time in minutes

(From Rydin H & Verney E B (1938) Quart J exp Physiol 27 343)

cacophonous noise or the stimulus of a weak subcutaneous faradic current (Fig 52) produced an inhibitory response quite similar to that observed as a result of exercise A study of the antidiuretic response to emotional stress alone was then undertaken and it was found that the response was unaltered by division of the renal nerves removal of the right adrenal gland and denervation of the left or abdominal sympathectomy From these and other experiments the conclusion was drawn that the

Feldberg and Vogt (1948) found very low values for the choline acetylase (enzyme system concerned with the formation of acetyl choline) content of the neurohypophysis though they obtained higher values for the supraoptic nuclei More recently Duke Pickford and Watt (1950) have been able to produce a reversible state of diabetes insipidus by injection of di isopropyl fluoro phosphate (D F P) an inhibitor of cholinesterase directly into the supraoptic nuclei of dogs This procedure resulted in first a profound inhibition of urine flow followed by a polyuria of about 4–19 days duration and then return of the urine volume to normal values It seems then that the cells of the supraoptic nucleus are under the control of at least some cholinergic afferent fibres but that the fibres of the supraopticohypophysial tract are not cholinergic in nature

The effect of adrenaline on the nervous reflex release of the antidiuretic hormone

It is an interesting fact that adrenaline seems to be related in some way with the nervous mechanism regulating the secretion of hormones from both lobes of the pituitary gland In earlier chapters the relationship between adrenaline and increased re lease of adrenocorticotrophic hormone and possibly with the release of gonadotrophic and thyrotrophic hormones have been discussed In this and the next chapter work dealing with the influence of adrenaline on the secretion of the neurohypophysis will be mentioned

As described above Verney and his colleagues established by means of a long series of experiments that emotional stress in the dog is followed by an inhibition of a water diuresis and that this inhibition is due to increased activity of the neurohypophysis elicited via the supraopticohypophysial nerve tract Much of this work had been performed on dogs after denervation of the kidneys and division of the splanchnic nerves Further study by O Connor and Verney (1945) revealed that in normal dogs emo tional stress may be followed by two different types of anti diuretic responses In 21 normal bitches emotional stress was followed by a long slow inhibitory response of posterior pituitary type in all tests in 3 animals by rapid fleeting inhibitory responses in all tests in 7 animals and by one or the other response or by combined responses in 11 animals In an attempt to separate

secondly different results might be expected from similar experiments performed on laboratory animals or man

It is unknown whether physical stress or trauma separate from any emotional stress or excitement would form an effective stimulus to the supraopticohypophysial system The observation of Rydin and Verney (1938)—that slow arterial haemorrhage (such as 58 c c blood loss in 5½ min) in the dog, results in trifling and transient changes in blood pressure but a profound antidiuresis of posterior pituitary type—would suggest that this is so However the reflex release of the vasopressor substance (or antidiuretic hormone) from the posterior pituitary gland might be looked upon as a compensating mechanism to maintain the blood pressure after haemorrhage Other stimuli that have been shown to result in posterior pituitary activation such as electro convulsive shock therapy in the human may clearly exert their effects in a number of ways It is difficult to devise experiments in which physical trauma may be imparted to an animal without at the same time producing emotional excitement

It is clear however that certain afferent nerve fibres end on and modify the activity of the supraoptic nuclei The anatomy of these fibres is unknown but there is evidence that at least some of these fibres are cholinergic in nature Pickford (1939) found that intravenous injection of acetylcholine into dogs inhibited a water diuresis, the response being abolished by removal of the neural lobe of the pituitary gland Since this effect of acetylcholine was exhibited in the presence of atropine it seemed likely to belong to those effects of acetylcholine called nicotine like and it was later found that nicotine itself had a similar action in the human (Burn, Truelove and Burn 1945) Pickford (1947) localized the site of action of acetylcholine more exactly by finding that injection of 0 004 ml (2–40 μg) of acetylcholine solution directly into the supraoptic nuclei of dogs resulted in liberation of the antidiuretic hormone Further evidence for a central action of acetylcholine is the fact that much smaller doses are effective if injected in the carotid artery rather than intravenously (Pickford and Watt 1951) It was then apparent that it was the cells of the supraoptic nucleus and probably not the cells (if any) upon which the supraopticohypophysial tract terminates that are sensitive to acetylcholine This is supported by the fact that

that adrenaline may exert its effect at least in part by an action as far downstream in the neural mechanism as the supraoptic nuclei

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the mechanisms by which these two types of responses are brought about it was found that removal of the neural lobe of the pituitary abolished or greatly diminished the slow type of inhibition, but left the rapid type of inhibition unchanged In other dogs the operation of abdominal sympathectomy splanchnic nerve section and renal denervation was followed by the unexpected result that emotional stress now invariably caused a long slow inhibition of water diuresis, whatever had been the nature of the inhibition before operation It seems therefore that there is some effect produced by the sympathetic nerve supply to the kidney and adrenal which not only produces a rapid fleeting inhibitory response after emotional stress but may also block the response due to the neurohypophysis To investigate this latter phenomenon dogs in which the renal adrenal area had been denervated were used In these animals emotional stress was invariably followed by a pituitary type antidiuresis but it was found that injection of 15 μg of adrenaline 30 seconds before the emotional stress stimulus resulted in a rapid type inhibition with abolition of the slow inhibition Since injection of adrenaline did not modify the effect of injected posterior pituitary extract the conclusion was drawn that adrenaline prevents the release of antidiuretic hormone during emotional stress but does not affect the action of antidiuretic hormone on the kidney Further experiments showed that tyramine has an action similar to adrenaline in this respect indicating that this action of adrenaline is not highly specific

The way in which a previous injection of adrenaline or tyramine acts to prevent the release of antidiuretic hormone following an emotional stimulus is not clear Verney (1947) discusses two possibilities—that the action is induced by an increased cerebral blood flow or through a specific interference in the chain of chemical reactions initiated in the nervous system by the emotional stimulus He concludes that from the evidence available the latter alternative seems the more likely It is tempting to suppose that the site of action of adrenaline is at the supraoptic nuclei Since there is good evidence that a cholinergic mechanism obtains at the supraoptic nuclei and since injection of adrenaline may prevent the release of antidiuretic hormone which normally follows the injection of acetylcholine (Duke and Pickford 1951) there would seem to be grounds for concluding

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CHAPTER 10

REGULATION OF THE SECRETION OF THE OXYTOCIC HORMONE

Since the early observations of Dale (1909) and Ott and Scott (1910) that administration of crude extracts of the posterior pituitary gland may result in contraction of the uterus and expulsion of milk from the mammary gland much attention has been given to the possibility that these actions represent physiological functions of the gland Evidence now available shows that the uterine and mammary activities of posterior pituitary extracts both reside in the same fraction referred to in the following pages as the oxytocic *hormone* The oxytocic compound synthesized by du Vigneaud Ressler Swan Roberts Katsoyannis and Gordon (1953) was found to possess the expected activity when tested on the rat and human uterus and human mammary gland

It seems likely that the neurohypophysis is excited by nervous reflex paths to secrete oxytocic hormone under at least three different conditions

(*a*) During parturition—distension of different parts of the reproductive tract (uterine cervix vagina) may excite nervous paths in the spinal cord and hypothalamus thus leading to oxytocic secretion and forceful uterine contractions

(*b*) During coitus—reflex excitation of oxytocic secretion may lead to increased uterine motility and so play a part in transportation of sperm

(*c*) During suckling—sensory stimulation of the nipple evokes reflex secretion of oxytocic hormone which causes contraction of the myoepithelial tissue of the mammary gland and so results in milk ejection

The data relating to these three possible functions of the neurohypophysis will now be discussed

1 The neurohypophysis and parturition

There has been a tacit assumption on the part of most authors ever since the oxytocic (quick birth) activity of posterior pituitary extracts was first discovered to assume that the neuro

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one animal as often as desired over a period of many months Since the vagina of the normal rabbit is too long to give access to the uterine cervices a preliminary operation was carried out in which the upper segment of the vagina bearing the uterine cervices was transplanted to the anterior abdominal wall In order to standardize the oestrous state and therefore the uterine reactivity ovariectomy was performed at the time of the transplantation operation and a tablet of stilboestrol di n butyrate implanted beneath the skin of the flank The routine procedure for recording uterine motility was to restrain the animals in a position of dorsal decubitus insert a balloon in one uterine horn and record the uterine movements on a kymograph The results obtained using these techniques were as follows The anoestrous or spayed rabbit shows a quiescent uterus The records obtained showed no rhythmic contractions and in many cases were straight lines The reactivity of this type of uterus to posterior lobe extract or to stimulation of the supraopticohypophysial tract was nil or minimal As the oestrous state developed either through the activity of the animal s own ovaries or by the slow absorption of a stilboestrol pellet spontaneous rhythmic contractions appeared in the uterine tracings and at the same time the uterus responded to injection of posterior lobe extracts or to stimulation of the supraopticohypophysial tract The detailed pattern of the response varied with the dose and type of extract (whole extract or purified oxytocic or pressor fractions) and with the strength of the stimulus (see Fig 53) In general it may be said that 15–20 seconds after intravenous injection of posterior pituitary extract or 20–30 seconds after starting electrical stimulation of the supraopticohypophysial tract a strong tetanic contraction of the uterus commenced This tetanus was maintained for about a minute and was followed by a gradual fall in tone over a period of 4–5 minutes with the reappearance of spontaneous contractions of increased frequency and amplitude The response to electrical excitation of the neurohypophysis could be matched very closely by injection of appropriate doses of posterior pituitary extract Maximal stimulation was found to be equivalent to the injection of 200–500 mU of the oxytocic fraction Repetition of an experiment on any one animal over a series of days gave reproducible results Gradation of the intensity of the stimulus to the supraopticohypophysial tract resulted in equivalent grada

ently normal labour To test this possibility rats suffering from diabetes insipidus following high pituitary stalk section had either the muscles of the abdominal wall denervated or the nerve supply of the uterus interrupted (Colfer and Harris unpublished) In some of these animals even these procedures did not prevent normal evacuation of the uterus Denervation of the abdominal wall often resulted in the last member of a litter being retained in the vagina the other members of the litter having been shunted into the external world by their next of kin

(*c*) *Stimulation of the neurohypophysis* In 1938 two papers appeared which indicated the hormonal nature of the oxytocic principle Chang Lim Lu Wang and Wang (1938) found that stimulation of the central end of the vagus in the dog caused the appearance of an oxytocic substance in the blood and Haterius and Ferguson (1938) found that electrical stimulation of the pituitary stalk of the rabbit resulted in increased uterine activity Haterius et al first used oestrous rabbits but found that their uterine reactions were too irregular They finally used post partum rabbits 2–8 hours after delivery The animals were anaesthetized the uterus filled with Ringer s solution and cannulated and the pituitary stalk stimulated with the aid of a stereotaxic instrument Such stimulation was shown to result in increased frequency and sometimes increased amplitude of the uterine contractions The response was abolished by cauterizing the pituitary stalk but not by spinal transection vagotomy or splanchnic nerve section This work was criticized on the grounds that the uterine responses may have been secondary to changes in blood pressure In reply to this objection further work by Ferguson (1941) showed that the uterine response was still obtained even if all the tissues of the neck were crushed except the carotid arteries jugular veins and a flap of skin Also any increase in blood pressure was found to follow the uterine response and not to precede it

More detailed studies of the uterine responses to stimulation of the supraopticohypophysial tract of the rabbit were made with the remote control technique of stimulation (Harris 1947—see Chapter 4) Using this method it became a relatively simple procedure to study the effect of stimulation of different areas in the hypothalamus or pituitary on the uterine motility of the conscious rabbit Repeated experiments could be performed on any

were all markedly decreased or disappeared It should be mentioned that in these experiments the stimulus was of a highly localized nature maximal responses were obtained from the uterus only if the stimulating tip of the electrode was in or in contact with some part of the supraopticohypophysial tract in the tuber cinereum or in the infundibular stem and no uterine response was observed if the electrode was more than 0 5 mm from the tract in any direction

The uterine reactions following stimulation of the pituitary stalk in the oestrous or oestrogenized rabbit (Harris 1947) were very similar to those described by Haterius and Ferguson (1938) and Ferguson (1941) in the post partum rabbit This fact is perhaps hardly surprising since the endocrine state of some post partum rodents is probably very similar to that of oestrus as shown by the regularity with which mice rats and non suckling post partum rabbits come into oestrus within a few days of delivery The fact that electrical stimulation of the neurohypophysis or its nerve supply results in secretion of the oxytocic hormone and thereby greatly augmented uterine activity cannot be taken as positive evidence that the neurohypophysis plays any part in normal labour though it demonstrates the potentiality of the gland to fulfil such a role However Ferguson (1941) produced further evidence relating to this problem In a study conducted on rabbits 8–48 hours after parturition he found that mechanical dilatation of the body of the uterus cervix of the uterus or vagina stimulates a nervous reflex release of oxytocic hormone and an increase in contractions of the body of the uterus That these reactions are mediated by an afferent nervous path was shown by the results of sectioning the spinal cord and that they involved the pituitary gland by the fact that hypophysectomy abolished the uterine responses Ferguson suggests that the mechanism of parturition involves reflex stimulation of oxytocic secretion probably in amounts varying with the part of the reproductive canal undergoing dilatation and postulated a series of reflexes that might form the basis for the delivery of a number of uterine foetuses one by one to the exterior Although this work was performed 13 years ago as far as is known it has not been repeated in the rabbit or other forms This seems unfortunate since such observations might be of much significance in regard to the mechanism of labour and since

tion of the uterine response, so that responses that matched with these following injection of 10 mU to 200–500 mU posterior pituitary extract could be produced at will During periods of pseudopregnancy or following injection of progesterone the spontaneous activity of the uterus and the responses to posterior pituitary extract or electrical excitation of the neurohypophysis

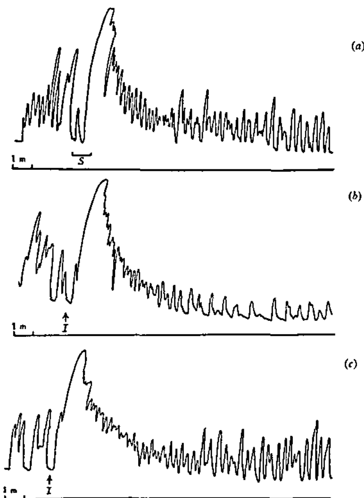


Fig 53 Response of the oestrous uterus to
(*a*) electrical stimulation of the supraopticohypophysial tract *S*
(*b*) intravenous injection of 500 mU pituitrin I
(*c*) intravenous injection of 500 mU pitocin I
(From Harris G W (1947) *Philos Trans B* 232 385)

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The uterine reactions following stimulation of the pituitary stalk in the oestrous or oestrogenized rabbit (Harris 1947) were very similar to those described by Haterius and Ferguson (1938) and Ferguson (1941) in the post partum rabbit This fact is perhaps hardly surprising since the endocrine state of some post partum rodents is probably very similar to that of oestrus as shown by the regularity with which mice rats and non suckling post partum rabbits come into oestrus within a few days of delivery The fact that electrical stimulation of the neurohypophysis or its nerve supply results in secretion of the oxytocic hormone and thereby greatly augmented uterine activity cannot be taken as positive evidence that the neurohypophysis plays any part in normal labour though it demonstrates the potentiality of the gland to fulfil such a role However Ferguson (1941) produced further evidence relating to this problem In a study conducted on rabbits 8–48 hours after parturition he found that mechanical dilatation of the body of the uterus cervix of the uterus or vagina stimulates a nervous reflex release of oxytocic hormone and an increase in contractions of the body of the uterus That these reactions are mediated by an afferent nervous path was shown by the results of sectioning the spinal cord and that they involved the pituitary gland by the fact that hypophysectomy abolished the uterine responses Ferguson suggests that the mechanism of parturition involves reflex stimulation of oxytocic secretion probably in amounts varying with the part of the reproductive canal undergoing dilatation and postulated a series of reflexes that might form the basis for the delivery of a number of uterine foetuses one by one to the exterior Although this work was performed 13 years ago as far as is known it has not been repeated in the rabbit or other forms This seems unfortunate since such observations might be of much significance in regard to the mechanism of labour and since

isolated observations continue to accumulate that the neurohypophysis is implicated It can now be taken as established that secretion of oxytocic hormone is excited by the sensory stimulus of suckling at the nipple and that this hormone is of prime importance in the process of milk ejection With this fact in mind the following two observations, for example would indicate a prominent role for the oxytocic hormone during labour in women

(i) Mavis Gunther (1948) has observed the expulsion of milk from the nipples of a woman in labour who was lactating from a previous pregnancy The mammary expulsion of milk coincided with the labour pains (uterine contractions) and was duplicated by injection of pituitrin at the end of the second stage of labour

(ii) The fact well known to midwives that uterine inertia complicating the birth of the second baby of twins may be cured by putting the first baby to the breast

2 The neurohypophysis and sperm transport

It is difficult at first sight to see any physiological significance in the fact that the rabbit possesses as described above a mechanism for increasing the activity of the empty oestrous uterus It is well established that the stimulus of coitus in the oestrous rabbit excites a nervous reflex activation of the adenohypophysis with consequent liberation of gonadotrophin On the basis of these facts it was suggested by Harris (1947) that coitus in the rabbit also by a nervous reflex excites the neurohypophysis to liberate oxytocin which increases the motility of the uterus and thus aids transport of the seminal fluid up the female reproductive tract

Rate of sperm transport There is good evidence that in many animals sperms reach the ovarian end of the Fallopian tube a very short time (few seconds or minutes) after mating In the rat Hartman and Ball (1930) found large numbers of spermatozoa were present in the uterus within 30 seconds of coitus and had reached the ovarian end of the uterus in less than 2 minutes The findings of other workers such as Blandau (1945) for the rat Florey and Walton (1932) for the rat and guinea pig and Evans (1933) for the dog also indicate that the speed of transport of sperm in the female tract is so rapid that it cannot be accounted for by the rate of swimming of the sperm and must be due in

some way to movements of the uterus and uterine tubes Similar findings have been put forward for the large agricultural animals such as the sheep and cow in which studies have been made recently in virtue of the interest aroused by techniques of artificial insemination The earlier accounts for the sheep and cow indicated that about 5 hours was required for sperm to reach the oviducts in both forms but more recent work using improved methods has demonstrated sperm in the ovarian portion of the uterine tubes in the ewe 6 minutes after mating (Starke 1949) and within 2 5–4 3 minutes after mating or artificial insemination of motile or non motile sperm in the cow (Van Demark and Moeller 1951) Data given by different workers for the rate of sperm transport in the rabbit have undergone the same sequence of change as in the case of the cow and sheep Heape (1905) found that sperm reach the ovarian end of the uterus about 2 hours after copulation in the rabbit Florey and Walton (1932) confirmed Heape s observation and asserted that in the case of the rabbit uterine movements play no part in the transport of seminal fluid However Parker (1931) after a thorough study of the time taken by sperm to pass from the vagina to the uterus in the rabbit concluded that this was too short to be accounted for by the rate of swimming of the sperm estimated at 0 05 mm /sec and from these and other observations concluded that the ascent of spermatozoa into the uterus is dependent on a vigorous muscular reaction of the uterus excited by the act of coitus Later studies by Krehbiel and Carstens (1939) have shown that spermatozoa may arrive at the utero tubal junction within 10 minutes from coitus in the rabbit

Two points clearly emerge from the above data Firstly that great care must be paid to details of technique in searching for sperm in different parts of the female reproductive tract Secondly that in the rat guinea pig dog rabbit sheep and cow sperm reach the upper end of the uterus within a few minutes from mating that is with a speed that cannot be accounted for in terms of sperm motility

Uterine motility and sperm transport Parker (1931) quotes one excerpt from an early paper by Heape (1898) It is not clear how the spermatozoa naturally find their way from the vagina to the uterus but from certain experiments which I have made on rabbits I am inclined to think the greater part of it is drawn in

by a sucking action of the uterus The os which is placed above *the ventral wall of the vagina appears to dip down into the midst* of the spermatozoa as they lie on the floor of the vagina and in conjunction with peristaltic contraction of the uterus to be with drawn again and this action appears to be repeated more than once at intervals This sucking action of the uterus in the rabbit above mentioned was induced by stimulating the erectile tissue of the vulva This last sentence of Heape s almost im *plies a reflex with the sensory receptors in the genital area* as underlying the increased uterine motility These results have been confirmed by the X-ray studies of Krehbiel and Carstens (1939) In this work it was found that artificial stimulation of the vulva of rabbits caused radio opaque fluids placed in the vagina to be carried to the tubal end of the uterus in 2-5 minutes

A similar activation of the uterus seems to be brought about *by the natural stimulus of mating Reynolds (1930) (his Table I)* noted that coitus in the rabbit results in increased uterine move ments However since such increased movements might or might not result in increased transport of the fluid up the genital tract the observations of Millar (1952) are of more significance in this respect Millar measured the intrauterine pressure in the mare by means of a cannula in the cervix and found that a con *siderable negative pressure is developed during coitus In the* two mares tested during mating 79 ml and 80 ml of fluid were sucked into the uterus respectively in approximately 5 seconds This required a pressure difference of 0 7 pound per square inch making allowance for atmospheric pressure

Evidence that the increased uterine movement during mating is of neurohypophysial origin has been obtained VanDemark and Hays (1952) inserted a balloon into the uterus of cows and recorded uterine movements during the several phases of mating They found that the sight of the male the approach mounting and final ejaculation all served as stimuli which resulted in in creased uterine tone and contractions *Ejaculation produced the* greatest uterine response which was characterized by tetanic contractions VanDemark and Hays (1951) also found that uterine motility was produced by stimulation of the vulva and cervix during artificial insemination in the cow These uterine motility patterns could be duplicated in the absence of other stimulation by injecting the cow with 15 units of oxytocin A

line of evidence that the increased uterine activity during coitus is causally related to release of oxytocic hormone is derived from observations on the mammary gland As mentioned above it is well substantiated that the main factor underlying milk ejection is the blood level of oxytocin With this point in mind it is significant that (*a*) manipulation of the vulva and cervix uteri of the cow results in a rise in intramammary pressure (Hays and VanDemark 1953) (*b*) the act of mating in the lactating human female may be followed by a flow of milk from the mammary glands (Pickles 1953 Harris and Pickles 1953 Campbell and Petersen 1953) Such milk ejection may occur independently of any manipulation of the mammary gland and may in certain individuals follow even trivial stimuli (a compliment by the husband)

So far no *direct* evidence is available linking the stimulus of coitus with oxytocin release There are no reports of blood assays of oxytocic hormone before or after coitus and no data regarding the rate of sperm transport in animals following destruction or denervation of the neurohypophysis The time appears appropriate for such experiments

3 The neurohypophysis and milk ejection

Lactation consists of two main processes milk formation in the mammary gland which is largely under the control of the adenohypophysis and milk ejection which is now known to be under the control of the neurohypophysis These two distinct processes should be borne clearly in mind especially when considering experiments concerning the hypothalamus or pituitary gland which may involve changes in secretory activity of either or both lobes of the pituitary and therefore in either or both parts of the lactational performance

The mechanism whereby suckling young obtain milk from the mammary gland has long been a matter of speculation As early as 1898 Schafer suggested three possible mechanisms that might underly this process He thought that the act of suckling might stimulate a nervous reflex in the mother resulting in (i) muscular contraction in the breast (ii) arterial dilatation in the breast or (iii) increased milk secretion Any theory advanced in this field must be able to account for the striking feature known as the let down in agricultural circles or the draught in medical circles The let down refers to the sudden rise in

by a sucking action of the uterus The os which is placed above the ventral wall of the vagina appears to dip down into the midst of the spermatozoa as they lie on the floor of the vagina and in conjunction with peristaltic contraction of the uterus to be with drawn again and this action appears to be repeated more than once at intervals This sucking action of the uterus in the rabbit above mentioned was induced by stimulating the erectile tissue of the vulva This last sentence of Heape s almost im plies a reflex with the sensory receptors in the genital area as underlying the increased uterine motility These results have been confirmed by the X ray studies of Krehbiel and Carstens (1939) In this work it was found that artificial stimulation of the vulva of rabbits caused radio opaque fluids placed in the vagina *to be carried to the tubal end of the uterus in 2–5 minutes*

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milk ejection a reflex in which the afferent side of the arc is formed by sensory nerves and pathways and the efferent side by the oxytocic hormone in the blood stream Evidence that a humoral component is involved was implied by the early and significant results of Gaines (1915) Five years after Ott and Scott (1910) showed that injection of pituitary extract increases the amount of milk obtainable from the cannulated udder of the goat Gaines showed that injection of posterior pituitary extract produces pressure changes in the cisterns of the goat s udder that are very similar to the changes observed in the udder of the cow during milking From studies on the dog Gaines also observed (*a*) the long latent period between the start of suckling by pups and the onset of milk flow and (*b*) the fact that suckling pups cannot obtain milk from an anaesthetized bitch unless the latter is injected with posterior pituitary extract It was left however to Ely and Petersen (1941) to put forward the view that secretion of oxytocic hormone stimulated by manipulation of the nipple forms an essential part of the physiological mechanism underlying the process of milk ejection Petersen and his co workers (see Petersen 1944) demonstrated that milk ejection could be stimulated in the isolated perfused udder by addition of oxytocin to the perfusing blood Similarly Petersen and Ludwick (1942) observed that blood withdrawn from the jugular vein of cows subjected to a milking stimulus but not from un stimulated cows evoked ejection of milk when perfused through the isolated udder preparation Confirmation of these results has been obtained by Peeters Massart and Coussens (1947) Such experiments established the humoral nature of the stimulus to the mammary gland but the origin of the humoral factor was not clear

If milk ejection is dependent on nervous excitation of the neurohypophysis it seemed possible that electrical stimulation of the nerve supply of this gland would elicit milk ejection and that lesions interrupting this nerve supply would interfere with milk ejection in response to normal suckling With these ideas in mind Cross and Harris (1952) investigated the effect of stimu lating and placing lesions in the supraopticohypophysial tract of lactating rabbits For the first experiment lactating rabbits were lightly anaesthetized and a fine glass cannula connected to a piston recorder inserted into the teat duct of one mammary gland The animal s head was then orientated in a stereotaxic

intramammary duct pressure and onset of milk flow that occurs about 30 seconds from the commencement of suckling or milking It is easy to observe in many forms (rabbit dog cow goat human and others) that during the first half minute of even vigorous suckling no milk is obtained by the young but that at the end of this period milk starts flowing suddenly and freely This long latent period between the commencement of suckling and milk flow would tend to eliminate theories based on a purely nervous reflex and contraction of muscle tissue

In the years that followed Schafer s account various workers found that at least two of the possibilities he had proposed could be eliminated Firstly Ely and Petersen (1941) showed that a nervous reflex in which both the afferent and efferent sides of the reflex are constituted by nerve pathways could not be the basis for the milk ejection process since cutting the motor nerves the ilio inguinal and posterior inguinal nerves to one half of the udder of the cow had no effect on the amount of milk obtained from that half of the udder Secondly histological examination of mammary tissue from many forms revealed a striking lack of muscular tissue especially around the alveoli Since alveolar milk as well as that in the ducts is made available to the young during suckling this paucity of muscle tissue in the breast gave rise to speculation as to the nature of the contractile cells Only recently has good evidence been obtained (Richardson 1949) that the contractile element is constituted by an elaborate system of myoepithelial cells which form basket like networks around individual alveoli and around the ducts And thirdly Gaines and Sanman (1927) demonstrated clearly that milk ejection could not be explained on the basis of the sudden formation of new milk stimulated by the act of suckling and resulting in overflow when they found that all the milk obtained at milking was present in the udder before the milking stimulus was applied

The suggestion that milk ejection is brought about by engorgement of mammary tissue with blood that a type of erection similar to that of the penis occurs in the udder has been developed by Hammond (1936) Hammond s excellent review should be referred to for an account of the work relative to this and other earlier theories

It is now clear that a neuro hormonal reflex is responsible for

neaud has shown that 100 units of ADH vasopressin have an activity on the mammary gland equivalent to that of 17–20 units of oxytocin. With this preparation of oxytocin the threshold dose was found to be only 1 mU. It is also of interest that *synthetic* oxytocin has been found active in milk ejection properties in human patients (du Vigneaud, Ressler, Swan, Roberts, Katsoyannis and Gordon (1953). A second comparison between the effects of stimulating the supraopticohypophysial tract electrically and the stimulus of normal suckling by a litter of young is of interest. Normally no milk is obtained for 30–90 seconds by a litter of vigorously suckling young. The total duration of a suckling period of a litter that has been previously separated from the doe is 2–5½ minutes and in this time they obtain 127 g ± 3·9 (S.E. of mean of 121 observations) of milk. In a study of this process in the rabbit Cross and Harris (1952) confirmed the old observation of Gaines (1915) that young cannot obtain milk from an anaesthetized mother. It was found that 6 young obtained no milk from an anaesthetized doe after 6 minutes vigorous sucking, but that after injection of 200 mU of posterior pituitary extract into the doe the litter obtained 20·8 g of milk in 2 minutes. However, comparing the response to the electrical stimulation in the lightly anaesthetized doe with that to suckling in the normal conscious rabbit we have the results shown in the following table.

	Milk ejection process observed after	
	(1) Stimulation of the supraoptico-hypophysial tract	(2) Normal suckling
Latent period	13–25 sec	30–90 sec
Duration of response	2–7 min	2–5½ min
Amount of milk ejected	0·3–1·6 ml from one teat duct [14·4–[illegible]6·8 ml from the 48 teat ducts usually present]	Mean 127 g

instrument and stimulation performed by inserting an electrode in a small trephine hole in the vault of the skull Stimulation of various regions of the ventral thalamus, hypothalamus and pitu itary gland was performed in 23 rabbits and from the results obtained it was clear that electrical stimulation of the supra opticohypophysial tract resulted in a copious discharge of milk into the vertically mounted cannula The stimulus used was a 50 cyc /sec sine wave alternating current that was found to have a spread at the voltage used of not more than ½ mm The characters of the responses were typically those of one humorally mediated The latent period was long 13–25 seconds, so that with a stimulus of ¼ minute duration the response often started after the end of the stimulus The peak of the responses occurred about 90 seconds after the beginning of the stimulus by which time 0 3–1 6 ml of milk had been ejected into the glass cannula The total duration of the responses was 2–7 minutes Com parison of the effects of stimulating the supraopticohypophysial tract with those seen following intravenous injection of posterior pituitary extract in the same rabbits showed that the effects of electrical stimulation have a slightly longer latent period 13–15 seconds as compared with 7–15 seconds A similar point was noted previously (Harris 1947) when the uterine responses to stimulation of the same nerve tract were compared to those fol lowing injection of extract It is likely that this discrepancy may be explained by the facts that (i) the pituitary secretion has to traverse a sinusoidal capillary plexus before reaching the jugular vein whereas the injected extract passes straight from the rela tively large marginal vein of the ear into the jugular circulation and (ii) that whereas the injection of extract was usually com pleted within a few seconds the period of stimulation and pre sumably of secretion was spread over ¼ minute or longer Apart from this difference in latent period the effects of electrical stimulation could be matched very closely by injection of appro priate doses (threshold dose 5 mU—dose producing maximal response 200 mU) of posterior pituitary extract The purified oxytocic fraction (Pitocin) was found to be more effective than the purified pressor fraction (Pitressin) in inducing milk ejection Later work by Whittlestone (1952) on the lactating sow and by Cross and van Dyke (1953) on the rabbit using highly purified polypeptide preparations supplied by du Vig

completely anaesthetizing the sacral region of the goat did not affect the result Milk ejection was also produced in this work by injecting blood obtained from the jugular vein of another stimulated animal

Further results bearing directly on the relationship of the neurohypophysis to milk ejection were noted incidentally by Harris and Jacobsohn (1952) during their study of pituitary grafts As described in Chapter 3 these workers transplanted pituitary tissue under the median eminence of the hypothalamus of hypophysectomized rats It was found that female rats bearing such transplants could become pregnant and deliver an apparently normal litter of living young at term However in the majority of cases the young died within a few days An examination of the young revealed a lack of milk in their stomachs although there was much milk present in the mammary glands of the mothers It was then thought that this might be accounted for by the lack of functioning neurohypophysial tissue in the graft Injections of posterior pituitary extract were tried and it was seen that such injection into the mother was followed by prompt withdrawal of milk by the young—as could be observed by examining the transparent abdominal wall of the young rat—and that repeated injections enabled the young to be reared These experiments demonstrated clearly the distinction between the dependence of milk secretion on the adenohypophysis and the dependence of milk ejection on the neurohypophysis

The relationship of the sympathetic system and adrenal medulla to milk ejection

There can be little doubt that the physiological mechanism of milk ejection involves sensory stimulation of the mammary area and probably various conditioned stimuli which by nervous pathways excite the supraopticohypophysial tract and neurohypophysis causing discharge of oxytocic hormone The oxytocic hormone is carried to the breast tissue causes contraction of the myoepithelial cells and thereby a rise in the milk pressure in the ducts and cisterns

It has long been known to clinicians that embarrassment worry or fright at the time of nursing may inhibit the flow of milk and the amount of milk obtained by the baby This aspect

The further experiments of Cross and Harris (1952) were concerned with the effect of lesions of the supraopticohypophysial tract on the lactational performance of the rabbit For this pur pose rabbits were allowed to remain with their litters for a period of 7-14 days after parturition They were then separated from the young but returned once a day for a suckling test the litters being weighed immediately before and after the suckling period and the milk yield determined After obtaining data regarding the normal performance of rabbits electrolytic lesions were placed in some part of the hypothalamus or pituitary gland using a stereotaxic instrument which involved a minimum of surgical trauma The effect of these lesions was studied in 10 rabbits that made good post-operative recoveries and in which appetite and nursing behaviour was not affected In 5 of these rabbits the amount of milk obtained by the litter during their daily suckling tests was not decreased after placing the lesions but even in creased slightly In these animals the lesions were found by histological study after death to be situated in the dorsal or posterior hypothalamic areas In the other 5 animals however the mean milk yield per daily suckling test before the lesions was 141 g whilst after placing the lesion a series of tests gave an average figure of 39 g This low figure of 39 g was obtained in spite of the fact that the mammary glands still contained much milk at the end of the nursing periods In other tests in these animals posterior pituitary extract was injected into the doe im mediately before suckling and the average milk yield found to be 119 g In these animals the lesions seen histologically had inter rupted the supraopticohypophysial tract in the median eminence or infundibular stem It would seem then that denervation of the neurohypophysis is incompatible with normal milk ejection but that in such animals replacement therapy with posterior pituitary extract is effective in restoring the milk yield towards the normal figure

At the time of the above work similar but independent re searches were being performed in Stockholm by Andersson (Andersson 1951*a* and *b*) Andersson recorded the outflow of milk from the teats of unanaesthetized sheep and goats following stimulation of the supraoptic nuclei by the Hess technique The humoral nature of the responses obtained was demonstrated by the fact that cutting the nerves to one side of the udder or by

tions in response to the amount of adrenaline liberated from the animal's own glands In support of this latter view could be quoted the findings of Ely and Petersen (1941) and Newton and Newton (1948) that injection of (large amounts of) oxytocic extract annulled the inhibition of milk ejection due to emotional stress Further work by Cross (1954 and personal communication) also supports this view Emotional stress was induced in lactating rabbits by restraint in dorsal decubitus This procedure blocked the milk ejection excited by the suckling of the young but the response was restored in most cases by injection of only 50 mU oxytocic extract

At the present time then it seems clear that emotional disturbance may block the milk ejection mechanism by discharging adrenaline from the adrenal medulla Although injected adrenaline may block milk ejection by an action on mammary tissue probably by causing vasoconstriction of the mammary blood vessels and so preventing access of oxytocic hormone to the contractile tissue it seems more likely that the adrenaline released by emotional stress acts centrally to prevent the secretion of oxytocic hormone

Clinical application

Failure of lactation in the human may occur for many reasons In view of the effects of emotional disturbances on milk ejection however it seems possible that this might be a common cause for poor milk yields Under such circumstances oxytocic extracts might be expected to be therapeutically beneficial in women in which the breasts contained milk in excess of that obtained by the baby Such a possibility has been tested by Hæger and Jacobsohn (1953) and found to be so Working in Lund Sweden these investigators observed the effect of injections of oxytocin on women with poor lactation and severe engorgement and pains in the breast 4 to 5 days after parturition Striking increases in milk yield were observed to follow an injection of oxytocic extract in all primiparous and most multiparous patients of this type It is also of interest that after one injection lactation proceeded normally again in all primiparous and most of the multiparous patients This latter observation is perhaps indicative of a psychological element present in the initial disorder Since such a simple procedure as injecting oxytocic extract may

has been studied more exactly in the human by Newton and Newton (1948) They found that the milk ejection process in a woman on a carefully controlled routine of nursing could be inhibited by causing her annoyance or discomfort Previous to this account Ely and Petersen (1941) had reported that frightening stimuli in the cow inhibited milk ejection and had suggested that this was due to sympathetic activity, since injection of adrenaline was found to have a similar effect Whittlestone (1951) has also found that painful faradic stimuli will prevent milk ejection in the cow

In considering sympathetico adrenal inhibition of milk ejection, there are two main possibilities Firstly the inhibition may be central that is circulating adrenaline may in some way prevent the release of oxytocic hormone In this connection there is the analogy from the work of Verney (see Chapter 9) that adrenaline may prevent the release of antidiuretic hormone from the neurohypophysis Secondly there is the possibility that the inhibition may be peripheral and that the mammary gland is prevented from reacting to sufficient amounts of oxytocic hormone in the blood stream These possibilities have recently been studied by Cross (1953) in the rabbit This worker found that (i) injection of 5–50 μg adrenaline intravenously into does before nursing interfered with milk ejection and that injection of an oxytocic extract immediately after the adrenaline did not restore normal milk ejection (ii) injection of 5–50 μg adrenaline into anaesthetized rabbits with cannulated teat ducts inhibited the milk ejection response to a subsequent injection of oxytocic extract or to stimulation of the supraopticohypophysial tract and (iii) stimulation of the sympathetic area of the posterior hypothalamus which produced signs of sympathetic activity such as pupillary dilatation and exophthalmos also inhibited the milk ejection response to injection of oxytocin extract Further studies have shown that bilateral adrenalectomy abolishes the inhibition produced by stimulation of the posterior hypothalamus (Cross personal communication) From these results it seems that adrenaline in the doses used does exert a peripheral effect in the mammary gland since it abolished the milk ejection to injection of oxytocic hormone However Cross is careful to discuss the possibility that a central inhibition may occur as well or may be the more important factor under physiological condi

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be of much use in treating a certain proportion of lactational failures and in preventing ineffective or prolonged suckling which may lead to cracked nipples and breast abscesses these observations should have considerable practical application

Summary

The factors responsible for exciting secretion of the oxytocic hormone appear to derive mainly from the external environment —tactile stimuli of the mammary glands vulva cervix uteri and probably conditioned stimuli It appears highly probable that these stimuli pass by afferent nerve pathways to activate the supraoptic nuclei in the hypothalamus which in turn excites the neurohypophysis Such reflex excitation of the neurohypophysis plays (i) a probable role in reinforcing the uterine contractions during parturition and in increasing the motility of the uterus during coitus and thus aiding sperm transport and (ii) a well established role in the process of milk ejection It is of much interest that secretion of adrenaline may modify reflex excitation of oxytocic secretion probably by a central nervous action

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CHAPTER 11

THE NATURE AND SITE OF FORMATION OF POSTERIOR PITUITARY HORMONE(S)

Few subjects in endocrinology have remained for so long such intriguing and tantalizing problems as those dealing with both the nature and the site of formation of posterior pituitary hormones

THE NATURE OF POSTERIOR PITUITARY HORMONE(S)

The phrase nature of posterior pituitary hormone(s) is one that requires a word of explanation The word hormone is freely used to refer to varying entities It has been used by various authors to refer to purified extracts obtainable from some endocrine glands to refer to the material manufactured by and stored in a gland and also to the substance secreted by a gland into the blood stream It is possible that in particular cases all these connotations of the word have the same meaning but it is also possible that what the chemist extracts what the gland forms and stores and what it liberates into the blood stream may be three different substances From the physiological point of view it would be more correct to retain the term hormone in the sense it was first used as a chemical messenger It would thus be kept to describe essentially the compound liberated by the gland cells into the blood

(*a*) Chemical analysis of posterior pituitary extracts

Following the original descriptions by Oliver and Schafer (1895) of the pressor activity of pituitary extracts and by Howell (1898) that this activity resided in extracts of the posterior lobe of the pituitary the idea was put forward that the pressor action observed was mediated via the adrenal medulla or alternatively that an adrenaline like substance was present in the posterior *pituitary gland A later view expressed by Abel and Kubota* (1919) was that posterior pituitary extracts owed their plain muscle stimulating activity to the presence of histamine This hypothesis was quickly disproven by the difference in chemical

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from the laboratory of du Vigneaud and his collaborators that will undoubtedly be referred to as classical in the years to come These workers first isolated what are probably the pure active principles using a method of countercurrent distribution between two appropriate immiscible solvents The apparently pure oxytocic principle was isolated as a solid of which (about) 1 25 μg = 1 unit (Livermore and du Vigneaud 1949 Pierce and du Vigneaud 1950*b*) as compared with the antidiuretic pressor extract which had an activity of (about) 2 2 μg = 1 unit (Turner Pierce and du Vigneaud 1951) The chemical nature of these two highly active polypeptides was investigated and it was found that each of them contains eight amino acids of which 6 are common to both (Pierce and du Vigneaud 1950*a* and *b* Turner et al 1951) The distribution of these ten amino acids are indicated in the accompanying table and compared with the data given by Block and van Dyke (1952) for their extracted protein

THE DISTRIBUTION OF AMINO ACIDS IN NEURAL LOBE PROTEIN AND IN OXYTOCIC AND ANTIDIURETIC PRINCIPLES

Component	Gram moles in 2 × 10 g of protein	*Molar ratio in*	
		Oxytocic principle	Antidiuretic vasopressor principle
Arginine	4	0	1 00
Phenylalanine	7	0	0 93
Tyrosine	4	0 97	0 94
Cystine	14–15	1 08	0 84
Leucine	11	1 00	0
Isoleucine	7	1 00	0
Aspartic acid	9–10	1 09	1 08
Glutamic acid	26	1 10	1 14
Glycine	34	1 09	1 09
Proline	15	1 02	1 13
Histidine	1*	—	—
Ammonia	27	3 28	2 97
Nitrogen	16 04	16 19	19 5
Sulphur	4 62	6 15	—

* Amino acid for reference

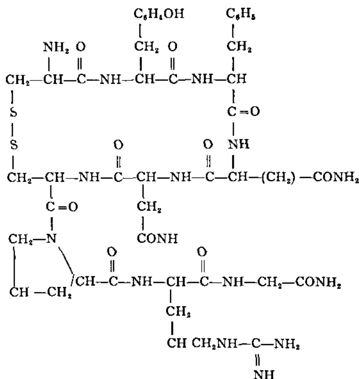
(From van Dyke (1953) *J Endocrinol*)

properties between histamine and the physiological active substance in the extracts It then became generally accepted that the activity of posterior pituitary extracts was due to specific hormones elaborated by the gland but from 1920 onwards there was much controversy as to whether the gland formed only one hormone with multiple activities or two or more hormones Abel and his co-workers (Abel Rouiller and Geiling 1923 Abel 1930) were enthusiastic advocates of the Unitarian theory on the grounds that the pressor and oxytocic activities of extracts disappear together when the extracts are submitted to heat tryptic digestion or the action of acids and alkalies and that pressor and oxytocic activities are in the same ratio in purified, as in crude extracts On the other hand Dudley (1919 1923) had managed to obtain a partial separation of pressor and oxytocic activity in various extracts Kamm Aldrich, Grote Rowe and Bugbee (1928) later separated fairly pure preparations of the oxytocic and antidiuretic pressor fractions For a review of the older literature reference should be made to the articles by Kamm et al (1928) and by Irving and du Vigneaud (1943)

In more recent years much attention has been given to the question of the chemical nature of the physiologically active substances in posterior pituitary extracts Van Dyke Chow Greep and Rothen (1942) extracted a single protein with the biological activities of both principles in about the same ratio as they are found in the posterior lobe The protein is free from the hormone of the pars intermedia which causes dispersion of the pigment in amphibian melanophores Its molecular weight is about 30 000 However the biological activity of the material was found to be low (1 unit = 61 μg) Although van Dyke et al produced considerable evidence that oxytocic and antidiuretic activities are present in constant ratios and made detailed chemical examination of the protein to establish its homogeneity the possibility of the adsorption of highly active principles on to an otherwise inert protein could not be finally excluded This protein isolated from fresh beef posterior lobes at 4 C by dilute acid and subsequent salting out at pH 3 90 contains a high percentage of cystine and all the common amino acids except cysteine, methionine hydroxyproline and tryptophan (Block and van Dyke 1952)

During the last four years a series of papers have appeared

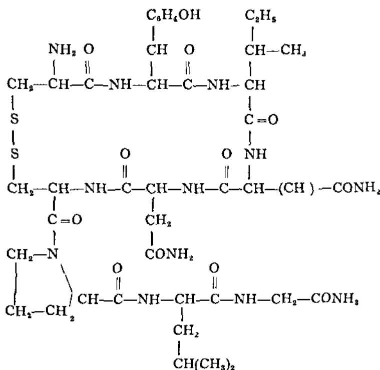
Similar work by du Vigneaud and his group (du Vigneaud Lawler and Popenoe 1953) on the antidiuretic pressor hormone has revealed the probable structure of this compound as—



Proposed structure for antidiuretic pressor hormone

Synthesis of this octapeptide structure has led to biologically active material The crude reaction product of the final step possessed both pressor and antidiuretic activity

Further work by du Vigneaud et al suggested the following formula for the oxytocic hormone (du Vigneaud Ressler, Swan, Roberts, Katsoyannis and Gordon 1953)—and also resulted in the



Oxytocic hormone

synthesis of this compound which was found to possess similar chemical and physiological properties (milk ejecting and uterine contracting activities) to natural oxytocin

exist such as that first suggested by van Dyke Chow Greep and Rothen (1942) that the neurohypophysis forms and stores a single parent substance of multiple activities from which various active side chains are split off differentially and secreted separately into the blood stream according to the requirements of the organism

The antidiuretic pressor oxytocic ratio in the glands of adult mammals of most species has been found to be 1 1 by the majority of workers This ratio has been reported for the guinea pig rabbit and cat (Simon and Kardos 1934) human (Simon and Nagy 1934 Heller and Zaimis 1949) rat and cat (Dicker and Tyler 1953*b*) More extensive studies by Macaulay Landgrebe and Waring (see Waring and Landgrebe 1950) have shown that the neurohypophysis of the rat horse sheep pig and cat has an antidiuretic pressor oxytocic ratio of 1 1 These workers assayed also the posterior pituitary of a single wallaby (found to have an antidiuretic pressor oxytocic ratio of 3 1) and the glands of a series of rabbits and guinea pigs These latter were found to have varying contents from one animal to another though there was never more oxytocin than vasopressin The comment is made that the ratio tends to approximate to a whole number of 1 1 2 1 or 3 1 Some observations by Dicker and Tyler (1953*b*) support the claim that the neurohypophysis of the guinea pig may have a lower oxytocin content relative to antidiuretic pressor than standard extracts In this respect then the glands of the wallaby guinea pig and rabbit may be similar to those of the whale (Geiling 1935) and armadillo (Oldham 1951) in which figures for the antidiuretic pressor oxytocic ratio have been given from 1 0 1 to 1 0 04

Some data are available for the activity ratios of the glands of animals belonging to the same species but under different physiological states

1 *New born animals* Comparison of the glands of new born animals with adults has shown that per mg tissue there is less extractable activity in the new born glands Heller (1947) and Heller and Zaimis (1949) claim that the anti diuretic pressor oxytocic ratio is still 1 1 in new born rats and humans whereas Dicker and Tyler (1953*a* and *b*) have found a greater proportion of antidiuretic pressor

One interesting pharmacological property of the highly purified extracts is that purified oxytocin possesses no pressor or antidiuretic activity but purified vasopressin possesses a slight degree of intrinsic oxytocic activity One hundred units of vasopressin possesses oxytocic activity equivalent to 5 units as determined by the isolated rat uterus assay 13–15 units determined by the chicken blood pressure depressor assay and 20 units by the milk ejection assay (see Popenoe Pierce du Vigneaud and van Dyke 1952)

If the oxytocic and antidiuretic pressor substances that have been synthesized *are in fact identical with the natural products* (as seems highly likely from the published data) this achievement of the du Vigneaud group will constitute the first synthesis of a polypeptide hormone and as they point out it will open *the door to investigating the effect of slight changes in the structure* of the compounds on their chemical physical and biological properties

(*b*) Data derived from assays of neurohypophysial extracts

It may be argued that knowledge of the antidiuretic pressor oxytocic ratio in the neurohypophysis of different forms or in the same form under different conditions would give information regarding the nature of the posterior pituitary secretion If the ratio of hormonal activities was found always to be 1 1 (as compared with standard extracts of cattle glands) it would suggest that a single molecule containing all activities was formed and secreted by the gland If, however the ratio was found to vary this might suggest the formation of two or more different molecules each with a specific activity and undergoing independent secretion In the latter case of varying hormonal ratios however other possibilities suggest themselves It is conceivable that the gland secretes a single substance of combined activities and that it forms this substance along a complicated metabolic path in which perhaps a side chain conferring antidiuretic pressor activity is added to a precursor compound before it acquires oxytocic activity Under these circumstances different glands might contain varying ratios of antidiuretic pressor oxytocic activities though the oxytocin would never exceed the antidiuretic pressor Clearly many other possible combinations

sensitive to permit such measurements to be made However approximate values for the antidiuretic pressor and oxytocic activities of the secretion have been obtained by studying the effect of the secretion on the organs of the animal itself

There is evidence that electrical stimulation of the supra opticohypophysial tract or the neurohypophysis directly elicits a secretion containing more oxytocic activity in relationship to antidiuretic pressor activity than whole standard posterior pitu itary extracts The data for this statement are as follows

(1) Ferguson (1941) noted in one experiment that stimulation of the pituitary stalk was more comparable in its propor tionate effects on the uterus and blood pressure of the anaesthetized animal to one U Pitocin (oxytocic ex tract) than one U Pituitrin (whole posterior pituitary extract) In other experiments he noted the uterine con traction elicited by stimulation of the stalk was qualita tively similar to that produced by injection of Pitocin He concluded that stimulation of the pituitary stalk liber-ates a hormone from the neurohypophysis with little pressor activity

The effect of different posterior pituitary extracts on uterine motility has been studied using ovariectomized rabbits implanted with a tablet of stilboestrol and in which the upper segment of the vagina carrying the uterine cervices has been transplanted to the anterior abdominal wall (Harris 1947 1948*b*) The uterine activity of such animals consists of irregular waves of contraction that appear at the rate of approximately one a minute Intra venous injection of Pitocin (Fig 54e) results in a marked contraction of the uterus that remains tetanic for about a minute followed by rhythmic contractions of increased amplitude and frequency Intravenous injection of Pit ressin (Fig 54c) results in a sudden short contraction of the uterus (perhaps due to a small amount of oxytocic impurity in the extract) followed by a diminution or abolition of all activity for a variable period Injection of mixtures of various doses of Pitocin and Pitressin (Figs 54b f g) was found to give the initial tetanic con traction (correlated with Pitocin) followed by an in

activity than oxytocic, in new born rats guinea pigs cats and dogs

2 *Dehydration or administration of hypertonic saline* According to Simon (1934) various procedures (muscular activity fasting adrenalectomy) do not alter the content of the rat s neurohypophysis whereas dehydration consequent on a reduced fluid intake results in a reduction of both anti diuretic pressor and oxytocic content of the gland simultaneously More recent studies have confirmed this earlier work Macaulay Landgrebe and Waring (see Macaulay 1950 Waring and Landgrebe 1950) have found in the rat that various stimuli such as electrical stimulation of the vagus nerve intravenous injection of 5 per cent NaCl 12 hours before killing and substitution of 3 per cent NaCl for drinking water for 11 days will all result in marked depletion of *both* antidiuretic pressor and oxytocic activities to equal extents

3 *Lactation* Since the suggestion that the milk ejection which occurs during suckling requires the secretion of oxytocic hormone in relatively large amounts the effect of lactation on the gland content of this substance has been investigated Macaulay (see Folley 1952) has studied the neurohypophysial content of antidiuretic pressor and oxytocic activities of goats killed just before and just after milking respectively No effect of milking could be detected on the content of either activity nor was there any difference between the values found in goats in milk and non lactating goats On the other hand Dicker and Tyler (1953*a*) found that the posterior pituitary of the lactating bitch possesses the same antidiuretic pressor content as the non lactating bitch but a much lower level of oxytocin content These latter results await confirmation

(*c*) The nature of the secretion elicited by electrical excitation of the neurohypophysis

It is clear that the conclusive evidence as to the nature of neurohypophysial hormone(s) will eventually be obtained from analyses of the hormonal content of pituitary venous blood in animals in different physiological states At the present time blood assays especially for oxytocic activity are not sufficiently

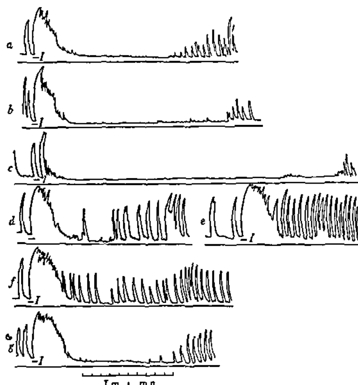


Fig 54 Tracings of kymograph recordings of the uterine responses of Rabbit 127 obtained from 19 6 47 to 24 6 47

- (*a*) Response to whole posterior pituitary extract I intravenous injection of 250 mU Pituitrin
- (*b*) Response to a mixture of equal parts oxytocic and pressor fractions I intravenous injection of a mixture of 250 mU Pitocin and 250 mU Pitressin
- (*c*) Response to intravenous injection I of 250 mU Pitressin
- (*d*) Response to electrical stimulation of the supraopticohypophysial tract
- (*e*) Response to intravenous injection of 250 mU Pitocin
- (*f*) Response to intravenous injection of a mixture of 250 mU Pitocin and 50 mU Pitressin
- (*g*) Response to intravenous injection of a mixture of 250 mU Pitocin and 100 mU Pitressin

The time scale is the same for all tracings Note the effect of stimulating the nerve supply of the neurohypophysis (*d*) approximate to responses shown in (*f*) and (*g*)

(From Harris G W unpublished)

hibitory phase (correlated with 'Pitressin'') Injection of equal amounts of Pitocin' and Pitressin (250 mU of each) gave a response similar to that following the injection of 250 mU of whole posterior pituitary extract ('Pituitrin' Fig 54a) In some rabbits it is thus possible to form a rough estimate of the proportion of oxytocic and pressor substances injected from the uterine response In such animals the effect of stimulating the supraoptico hypophysial tract electrically (by the remote control method) was compared with the effects of injecting extracts and as may be seen from Fig 54d it appears that the stimulation elicited a secretion from the neurohypophysis equivalent in uterine effects to a mixture of 250 mU Pitocin and 50–100 mU Pitressin'

(ii) Simultaneous measurements of oxytocic and antidiuretic responses in the rabbit showed that electrical stimulation of the supraopticohypophysial tract elicited a secretion from the neurohypophysis that produced a greater oxytocic response relative to the antidiuretic than did injection of whole posterior pituitary extract (Harris 1947)

(iii) *In a series of rabbits a standard one minute stimulation* of the supraopticohypophysial tract was shown to produce an oxytocic effect equivalent to that produced by 200–500 mU of posterior pituitary extract Similar stimulation on the same rabbits was shown to produce an effect on intestinal peristalsis and blood pressure less than that evoked by 100 mU of posterior pituitary extract (Harris 1948*b*) It may be mentioned that there is good evidence in the rabbit that the peristalsis stimulating action of posterior pituitary extract is due to the pressor fraction

(iv) Intravenous injection of posterior pituitary extract or electrical stimulation of the supraopticohypophysial tract in the rabbit causes the appearance of an antidiuretic substance in the urine In rabbits in which one minute stimulation had been shown to elicit an oxytocic effect equivalent of 200–500 mU of posterior pituitary extract a similar stimulus was found to cause the appearance of less antidiuretic substance in the urine than intravenous injection of 100 mU Pituitrin (Harris 1948*a*)

sodium chloride solution given was 20 to 60 c c Control injections of 40 c c Ringer solution had no effect That the response was not due to concurrent emotional stress was shown by the fact that injection of hypertonic NaCl solution into a nembutal anaesthetized animal produced a similar response to that obtained before anaesthesia

Abrahams and Pickford investigated the effect of intracarotid injection of hypertonic saline in causing oxytocic hormone release in the dog but used the uterus as an end organ indicator of such release In ovariectomized bitches in which a uterine fistula had been prepared they found that emotional stimuli and also intracarotid or intravenous injections of concentrated NaCl solution caused not only an inhibition of water diuresis but a simultaneous and coterminous increase in uterine activity The antidiuretic effect could be matched by the intravenous injection of for example 3 mU Pitressin but an equivalent increase in uterine activity needed 80–100 mU Pitocin These experiments again indicate a preponderance of oxytocic hormone in the secretion liberated from the gland It is of great interest that section of the supraopticohypophysial tract in one bitch demonstrated a parallelism between the renal and uterine effects During the phase of primary polyuria the normal uterine activity disappeared (although the uterus still responded to injection of Pitocin) the activity reappeared in the normal interphase but disappeared again with the onset of permanent polyuria

It appears then that intracarotid injection of hypertonic saline results in release not only of the antidiuretic hormone but also of the oxytocic hormone as shown by the ensuing effects on the mammary glands and uterus

(ii) *Suckling or milking* There can be little doubt that the act of suckling or milking excites a nervous reflex release of oxytocin Several groups of workers have now investigated the effect of suckling or milking on the urine flow of hydrated animals The results obtained are uniform in that small antidiuretic effects have been observed Peeters Coussens Bouckaert and Ovaert (1949) and Peeters and Coussens (1950) found that milking in the cow resulted in an inhibition of a water diuresis which was simulated by intravenous injection of 10 mU posterior pituitary extract Cross (1951) observed a slight antidiuresis to follow suckling in rabbits In 49 out of 55 experiments the urine flow

It is clear then that electrical stimulation of the supraoptico hypophysial tract in the conscious rabbit evokes the liberation from the neurohypophysis of a secretion which possesses less antidiuretic pressor activity in proportion to its oxytocic activity than whole posterior pituitary extracts (with their 1 1 ratio by definition)

(*d*) The nature of the secretion elicited by reflex excitation of the neurohypophysis

In recent years it has been firmly established that an increase in the osmotic pressure of the carotid blood results in increased release of the antidiuretic hormone from the neurohypophysis and that the act of suckling reflexly stimulates the release of oxytocic hormone There is also evidence that coitus excites oxytocic secretion It is possible to envisage satisfactory purposeful reasons why the above stimuli should cause secretion of the antidiuretic or oxytocic hormones—to conserve body water during conditions of dehydration and to cause milk ejection and increased uterine motility during suckling and coitus However if the neurohypophysis liberates two separate hormones into the blood stream there would appear to be little reason to suppose that these stimuli would individually result in increased secretion of both hormones On the other hand if the neurohypophysis liberates one hormone with multiple activities then each of the above stimuli would result in simultaneous antidiuretic and oxytocic effects Some data regarding the hormonal activities released by these stimuli are now available

(1) *Increased osmotic pressure of carotid blood* The beautiful analysis by Verney (Chapter 9) has established the fact that an increase in the osmotic pressure of the carotid blood will result in discharge of the antidiuretic hormone from the neurohypophysis The effects of intracarotid injection of hypertonic sodium chloride solution on the possible release of oxytocic hormone has now been studied by Andersson (1951) who observed the effects produced on the mammary gland in the goat and by Abrahams and Pickford (1953) who studied the uterine effects in the dog Andersson found that injection of strongly hypertonic (0 684–1 368 M) solutions of sodium chloride into the right common carotid artery resulted in milk ejection if the rate of administering the saline was rapid (2 c c /sec or faster) The volume of

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tion of the paraventricular hypothalamic nuclei in the rat results in loss of extractable oxytocic material from the neurohypo physis A posterior lobe completely lacking oxytocin showed a normal pressor content Cross (personal communication) has found that electrical stimulation of the paraventricular nucleus in rabbits may result in a marked milk ejection response These results have so far only appeared in preliminary form but they seem to constitute highly significant information regarding the physiology of the posterior pituitary gland

(*f*) Conclusions

At the present time there is insufficient evidence to state categorically whether the neurohypophysis secretes one hormone with multiple activities or two or more hormones each with separate activities

The chemical extraction of the gland the purification of such extracts and latterly the analysis of the chemical structure of extracts and the synthesis of biologically active substances though undoubtedly a major and fundamental work in both bio chemical and endocrinological fields yield little information as to the nature of the hormone(s) liberated into the blood stream The polypeptides of du Vigneaud may in fact represent two hor mones or they may represent only two active side chains nor mally linked with a larger protein molecule

The information that electrical stimulation of the supraoptico hypophysial tract causes hormonal release with both antidiuretic pressor and oxytocic activity again throws little light on the prob lem The supraopticohypophysial tract may consist of several different fibre systems—each system being concerned with the regulation of a neurohypophysial hormone Alternatively the fibres of the supraopticohypophysial tract may be concerned with the regulation of the secretion of a single hormone that combines all neurohypophysial functions In either case electrical stimula tion of the tract would be expected to discharge all hormonal activities It is possible as suggested by the work of Cross that localized stimulation of different regions in the hypothalamus excites an individual component of the supraopticohypophysial tract and results in liberation of only one hormonal activity In this case the picture is much clearer From the present results however it may be concluded that if the neurohypophysis liber

was diminished and the concentration of chlorides in the urine increased by the suckling stimulus The antidiuretic responses in these rabbits could be duplicated by intravenous injection of 0 4–1 0 mU posterior pituitary extract The effect of suckling in lactating women has been studied by Kalliala and Karvonen (1951) In one series of women studied 13 antidiuretic responses occurred in the course of 23 suckling tests though in an equal number of control experiments (without suckling) an antidiuresis was seen on only 3 occasions Repeating this work on the dog Kalliala Karvonen and Leppanen (1952) found a constant antidiuretic response and an increase in sodium excretion to follow nursing Again however the response was slight being comparable to that produced by 0 01–0 5 mU posterior pituitary extract

It is clear then that slight antidiuretic effects are associated with a suckling stimulus and its accompanying milk ejection reflex The antidiureses observed are of the order of those produced by 0 5 mU posterior pituitary extract in the rabbit and dog and 4 mU in the cow It is of interest to compare these figures with those of 50–100 mU of oxytocic hormone necessary for normal milk ejection in the rabbit with the neurohypophysis denervated (Cross and Harris 1952) It is unknown how much oxytocic hormone is liberated by normal suckling in the rabbit but it is likely to be of this order of magnitude

(iii) *Coitus* From the evidence discussed in Chapter 10 it seems likely that coitus results in reflex release of oxytocic hormone Experiments have now been performed to see the effect of coitus on a water diuresis in the rabbit rat and human Cross (1951) found coitus in the rabbit had no effect on a water diuresis On the other hand Eranko Friberg and Karvonen (1953) reported that a slight antidiuresis occurred in rats that were exposed to the opposite sex and attempted copulation 4 times or more Similarly Friberg (1953) observed a significant antidiuretic effect to follow coitus in a male and female human subject These responses were of a posterior pituitary type though they were not assayed against injections of posterior pituitary extract

(*e*) The effect of hypothalamic lesions on extracts of the neurohypophysis

Olivecrona (1954) has recently reported that bilateral destruc-

(1932) who described parasympathetic activation on injection of posterior pituitary extract into the lateral ventricle of the brain in the human and many other workers who claimed to have found posterior pituitary principles in cerebrospinal fluid Later work using more highly purified preparations disproved the claims of Cushing and more accurately controlled assays of cerebrospinal fluid failed to detect posterior pituitary hormone in the fluid

It is now clear that the active principles are formed in the neurohypophysis and not in the pars intermedia Firstly in some forms such as the whale and Indian elephant the pars intermedia and pars distalis are separated from the neural lobe by a thick connective tissue septum and yet the neurohypophysis still contains the active extractable compounds The only difference in hormone distribution in these glands is that the melanophore dilating hormone is found in the anterior lobe (DeLawder Tarr and Geiling 1934 Oldham 1938 Oldham McCleery and Geiling 1938 Geiling Vos and Oldham 1940) Secondly the atrophic neural lobes of cats in which lesions had been placed in the supraopticohypophysial tract were found to have a very low content of antidiuretic pressor and oxytocic activities (Fisher Ingram and Ranson 1938) In these animals the pars intermedia was histologically normal and the gland still contained melanophore dilating hormone And thirdly it may also be mentioned that electrical stimulation of the pars intermedia is not so effective in causing an antidiuretic or oxytocic response in the rabbit as is direct stimulation of the neurohypophysis (Harris 1947)

In 1930 Bucy described cells in the neurohypophysis which he called pituicytes For some years it was thought that these cells might constitute the secretory glandular element of the neurohypophysis Gersh (1939) described osmiophilic droplets in these cells which he called the parenchymatous glandular cells He claimed that the cytology of the cells varied with the physiological state of the animal and in particular with the state of hydration This evidence was questioned by Hickey Hare and Hare (1941) and there would seem to be little reason for believing that the glandular cells of Gersh constitute a secretory mechanism

Perivascular terminations to the nerve fibres of the hypothalamo hypophysial tract have been described by many authors

ates only one hormone it differs in its physiological activity from that of standard posterior pituitary extract in that it has a greater oxytocic content relative to the antidiuretic pressor content than has the extract

The data gained from experiments in which physiological stimuli have been used to excite the gland reflexly are more informative Both the study of the hormonal depletion of the gland and of the hormonal activities released indicate that the neurohypophysis liberates antidiuretic pressor and oxytocic hormone(s), simultaneously under a variety of conditions This line of work also indicates that the oxytocic substance is released in relatively greater amounts than the antidiuretic pressor However several problems are raised by these results Firstly it may be argued that a strong enough stimulus of any type although it acts primarily in causing secretion of one hormone flushes out the other On this line of reasoning however it is difficult to understand why hypertonic saline causes the release of such apparent excess of oxytocin Secondly the suggestion may be made that the ratio of oxytocic antidiuretic pressor activities released by electrical stimulation of the neurohypophysis has been put at about 4 1 by intracarotid injection of hypertonic saline at about 30 1, and by suckling at about 100 1 and that *these discrepancies indicate that different stimuli release varying* amounts of two distinct hormones It may also be however that technical inaccuracy is responsible for the differing ratios

THE SITE OF FORMATION OF POSTERIOR PITUITARY HORMONES

Ever since hormonal activity was first associated with the posterior pituitary gland speculation has existed concerning the nature of the tissue responsible for forming the biologically potent material extractable from the gland

The lack of any obvious glandular cells in the posterior pituitary lead Herring (1908) to put forward the view that the active material in the posterior lobe is derived from the basophile cells of the pars intermedia which migrate posteriorly into the neurohypophysis and become transformed into hyaline masses These hyaline bodies (or Herring bodies) were thought to travel up the pituitary stalk be liberated into the cerebrospinal fluid in the third ventricle and to exert their actions by affecting nerve centres in the hypothalamus This view received the support of Cushing

nerve fibres was the histological representation of the hormone However it now seems clear that the stainable material is distinct from posterior pituitary hormones for the following reasons

(i) A mixture of absolute alcohol and chloroform will extract the stainable material from the neurohypophysis of the pig but leaves the hormonal concentration in the tissue constant (Hild and Zetler 1953) It is suggested that the stainable material is lipoid in nature (These findings have however recently been questioned by Sloper 1954)

(ii) Extracts of the hypothalamus have been purified by paper chromatography and the eluates assayed for posterior pituitary activity (Vogt 1953) It was found that the oxytocic activity of such extracts was only about 7 per cent of the pressor and antidiuretic activities Since pure antidiuretic pressor substance is known to possess some oxytocic activity the substance present in the hypothalamic extracts may be pure antidiuretic pressor hormone The hormonal substance present in the hypothalamus then is not the same as that in the neurohypophysis If the hypothalamic nuclei are responsible for forming antidiuretic pressor hormone the oxytocic hormone is either added during transport through the hypothalamus or in the neural lobe itself

(iii) Wingstrand (1953) has studied the time of appearance of the Gomori stainable material and of posterior pituitary hormones in the neurohypophysis of the embryo chick The antidiuretic activity in the hypothalamus was distinct in embryos incubated for ten days or more but the Gomori stainable material did not appear until the 13th or 14th day

The above observations have given rise to the view that the stainable material is intimately related to the intra axonal transport of the hormone as a carrier but is in fact a chemically distinct compound

The main evidence that the hormone is functionally correlated with the stainable material derives from experiments which show a corresponding distribution of the two in a variety of animals and under a number of different experimental conditions A

(Hair 1938, Vazquez-Lopez 1942 Green 1951 and others) The findings of Bodian (1951) in the opossum have drawn particular attention to the vascular relations of nerve terminals in the infundibular process Since the histological studies have revealed (*a*) no obvious secretory cells in the neurohypophysis and (*b*) the close relation of nerve fibres to blood vessels in this part of the gland much interest has centred around the idea of a neurosecretory process as underlying the formation and secretion of posterior pituitary hormones

Neurosecretion

Ernst Scharrer and other workers have, for many years discussed the possibility that certain nerve cells in the central nervous system secrete hormones into the blood stream In 1949 Bargmann demonstrated that the nucleus preopticus of fishes and amphibians (equivalent evolutionarily to the nuclei supraopticus and paraventricularis of higher vertebrates) stain selectively with Gomori s chrome haematoxylin method Since the original observation many workers have used this staining method to delimit not only the supraoptic and paraventricular nuclei but also the nerve tracts which pass from these nuclei into the neurohypophysis Beautiful histological preparations have been obtained in many vertebrates including such mammals as the rat rabbit mouse cat dog ox pig monkey and man (see Palay 1953 and Zetler and Hild 1954) in which these cell groups and fibres are stained in striking contrast to the surrounding tissues Only a few other regions of the central nervous system such as the subcommissural organ and some microglial cells (Wingstrand 1953 Wislocki and Leduc 1952) stain by this technique

It has been known for some time (Abel 1924 Melville and Hare 1945) that antidiuretic hormone may be present in extracts of hypothalamic tissue This fact together with the discovery of Bargmann made it seem possible that posterior pituitary hormones were formed in the supraoptic and paraventricular nuclei of the hypothalamus transported down the axones of the hypothalamo hypophysial tract and liberated into the blood vessels of the neural lobe of the pituitary (Bargmann and Scharrer 1951 Scharrer and Scharrer 1954) It was also suggested that the Gomori stainable material in these nuclei and

in the hypothalamus although the low concentration of the oxytocic hormone in this region of the brain requires further investigation

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close parallelism was found between the amount of neurosecretory material and the hormone content of the hypothalamus and neurohypophysis of the dog ox pig and man by Hild and Zetler (1952*a* and *b*) The highest concentration of the material and hormones was found in the dog the lowest in man Several workers have studied the effect of conditions of dehydration known to stimulate liberation of the antidiuretic hormone on the histological picture of the Gomori material A recent study by Leveque and Scharrer (1953) which gives references to previous work may be consulted During dehydration produced by withholding drinking water or by substituting hypertonic saline for drinking water the Gomori stainable material becomes depleted but returns after water is again supplied Under these conditions there is a parallelism between the liberation of antidiuretic hormone and the content of the Gomori material Under another set of conditions namely after pituitary stalk section Hild and Zetler (1953) claim a further correspondence Following such a procedure in the dog the hormonal and neurosecretory content of the neural lobe decreased but on the hypothalamic side of the section both increased above control values

Various rather wide sweeping statements have been made at different times by the protagonists of the neurosecretory hypothesis It has been stated that diabetes insipidus does not occur unless a lesion is placed in the immediate vicinity of the supraoptic nucleus that following stalk section signs of regeneration of a neural lobe may occur from the hypothalamic stalk stump and in the paper by Leveque and Scharrer (1953) the statement appears A critical analysis of the evidence on which Ranson s theory of the function of the posterior pituitary was based leads one to the conclusion that this theory should be abandoned in favour of the concept of the neurosecretory origin of the antidiuretic hormone Such claims as these which run contrary to a great deal of established data should be taken with reserve Many of the views of Ranson are undoubtedly true whatever the site of formation of posterior pituitary hormone turns out to be

At the moment the neurosecretory origin of the posterior lobe hormones remains an attractive hypothesis It offers a clear explanation for the lack of secretory elements in the neurohypophysis and for the presence of the antidiuretic pressor substance

in the hypothalamus although the low concentration of the oxytocic hormone in this region of the brain requires further investigation

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CHAPTER 1

MATURATION OF THE HYPOTHALAMO HYPOPHYSIAL MECHANISM

If as mentioned previously the function of the pituitary stalk is to form the connecting link between the external environment and many of the endocrine glands the question may well be asked at what stage of the pre neo or post natal life the function of the stalk commences There is now much evidence that the endocrine glands are in a state of functional activity at least towards the end of foetal existence (for a review of this subject see Jost 1953) During this period the external environment of the foetus is stabilized and although a reciprocal relationship between the secretion of for example the adrenocorticotrophic hormone and the blood concentration of adrenal cortical hormone (Wells 1948 Kitchell and Wells 1952) has been established it might be argued that the mechanism relating endocrine activity to changes in the external environment would not be developed until after birth The available data indicate that this is so

(*a*) *Onset of activity of the hypothalamus neurohypophysis* New born animals are unable to concentrate their urine to the same degree as adults when fluid intake is restricted (McCance and Young 1941 Heller 1949) One possible cause of this phenomenon is lack of development of the neurohypophysial renal mechanism which in the adult is a major determinant of the final concentration of the urine This mechanism might differ at the renal level so far as the sensitivity of the renal tubules to circulating antidiuretic hormone is concerned and/or might differ at the hypothalamic pituitary level in regard to the release of antidiuretic hormone produced by changes in the osmotic pressure of the blood It seems likely that both these factors play a part for it has been found that the kidney of the new born animal is less sensitive to injection of antidiuretic hormone (Heller 1944 and 1952) and also that the neurohypophysis contains considerably less antidiuretic hormone (calculated per 100 g body weight or per mg dry posterior pituitary tissue) than does the

adult gland (Heller 1947, Heller and Zaimis 1949) In accordance with this latter finding is the fact that the stainable neurosecretory material in the rat s neurohypophysis does not appear until after birth (Dawson 1953) It would be of much interest to compare the sensitivity of the hypothalamus neurohypophysis to nervous reflex excitation (for example to a pain stimulus) using the techniques available for assaying the blood concentration of antidiuretic hormone

(*b*) *Onset of activity in the hypothalamic anterior pituitary adrenocortical system* The well known effect of stress in discharging A C T H from the anterior pituitary gland of adult animals does not obtain in the new-born Jailer (1950) found in a study of the adrenocortical stress responses in new born and young rats that injection of adrenaline does not excite pituitary discharge of A C T H until the 8th day of life and that cold exposure (5 C for 75–150 minutes) was ineffective till the 16th day He suggests that one possible cause for these results is lack of development of hypothalamic nervous pathways The effect of stress on new born mice was studied by Thompson and Blount (1954) who observed that although injection of A C T H or adrenaline resulted in eosinopenia in mice less than one day old the stress of heat did not elicit this response till about the 11th day of life These workers suggest that the failure of new-born animals to respond to heat stimulation may be due to a pre pituitary break in the chain between the reception of the heat stimulus and the discharge of A C T H In studies on the human Jailer Wong and Engle (1951) found that whereas most full term infants responded to injection of adrenaline with an eosinopenia premature infants did not respond until 9–14 days after birth Control injections with A C T H made it seem unlikely that this was due to lack of sensitivity of the target organs In the present context it would be of interest to see the effect on the new born human of a stimulus acting through the nervous system

(*c*) *Onset of activity in the hypothalamic anterior pituitary gonad system* As discussed in Chapter 4 the onset of activity of the gonads which occurs at puberty seems to be in some way dependent on development of the nervous system rather than maturation of the anterior pituitary gland or gonad

The evidence suggests that although the activity of many endocrine glands starts during foetal life the mechanism whereby this activity is affected by environmental influences does not mature until after birth

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CHAPTER 13

HORMONES AND BEHAVIOUR

In the preceding chapters attention has been turned to the evidence dealing with the control exerted by the central nervous system over endocrine activity and to the feedback effects exerted by the target organ glands on the central nervous system (and/or the pituitary gland) whereby they indirectly regulate their own activity (see Fig 1) Another major effect of hormones on the nervous system is the regulation of patterns of behaviour appropriate to the blood concentration of circulating hormone

Hormones and patterns of behaviour

(*a*) *Gonadal hormones* The effect of varied concentrations of a hormone in the blood stream resulting in varied patterns of behaviour is most clearly seen in relation to the sex cycles of the lower mammals As is well known female mammals will only accept the male during a limited part of the oestrous cycle that is during the period known as oestrus' Corner (1946) has discussed the derivation of the term oestrus , from the Latin meaning a gadfly It had been observed that periods of frenzy occurred in cattle attacked by the gadfly, so that the term oestrum (Latin neuter form) came to mean any condition of periodic frenzy and oestrus (masculine form) was later used by Walter Heape (1901) to describe the recurrent periods of heat seen in female mammals In its original use then the term 'oestrus denoted a certain behaviour It is now possible to correlate this particular type of behaviour with the blood concentration of circulating ovarian hormones

In any species the behavioural pattern observed before during and after coitus is stereotyped In the presence of a male the female rat in oestrus makes short darting runs ending in a crouching position tends to hop wiggles her ears and assumes a position of lordosis when mounted by the male (thus facilitating intromission) Certain aspects of this behaviour may be graded and measured For example Beach (1943) has defined a Copulatory Quotient as the number of lordosis responses divided by

the number of times the female was mounted multiplied by a hundred and has used this quotient to measure the behavioural changes in female rats following removal of various parts of the cerebral cortex The behaviour of the female guinea pig in oestrus as described by Young Dempsey and Myers (1935) is similar to that of the rat On being mounted by the male this animal adopts a position of lordosis with elevation of the pelvis and emits a characteristic hoarse purring noise The female rabbit responds very simply to attempted coitus If receptive she crouches elevates the pelvis and tail and thereby aids entry If non receptive she crouches but depresses or flexes the pelvis and tail thereby preventing entry The oestrous female cat possesses an extensive behavioural pattern that lends itself well to experimental study Bard (1940) has described courtship phenomena which serve to attract the male such as rolling excessive rubbing a low vocalization crouching and treading During intromission augmented treading is accompanied by a loud cry or growl The after reaction consists of more or less frantic rubbing squirming licking and rolling

Since the full picture of mating behaviour is not seen in the isolated animal whether in oestrus or not it is clear that sensory stimuli usually supplied by the presence of the male are necessary to trigger reflex mating patterns A certain blood level of gonadal hormone appears to be necessary to sensitize the central nervous system and when this is present particular sensory stimuli result in a particular pattern of behaviour As discussed below a variety of stimuli are effective in eliciting such behaviour and it should be noted that no single stimulus is essential for in the absence of one source others may be sufficient to evoke this behaviour

(*b*) *Thyroid and adrenal cortical hormones* It is as yet impossible to say that the hormones derived from the thyroid or adrenal cortex exert any effects over the behaviour of the normal animal Clear abnormalities in behaviour may result from diminished or increased production of these hormones but such effects may be secondary to disorders of general or nervous metabolism and it is therefore not safe at present to conclude that they play any part in the direct regulation of normal behaviour

Studies on lower animals in which the emotional state the ability to learn or the general motor activity of the animal has

(i) *The effect of hormones on specific morphological structures*

This aspect of hormonal action is clearly seen in the reproductive system The development of the reproductive tract and external genitalia under the influence of gonadal hormones might be supposed to be a necessary step in the development of oestrous or mating behaviour on the grounds that an increase in afferent nervous impulses from the genital tract is responsible for such behaviour That these afferent impulses are not necessary for fully developed mating behaviour is shown by the work of Josephine Ball (1934) who found that normal sexual behaviour occurs in female rats after removal of the uterus and vagina Similarly Bard (1935) showed that female cats subjected to abdominal sympathectomy and removal of the sacral region of the spinal cord can show typical oestrous behaviour and Root and Bard (1937) showed that the sexual aggressiveness of male cats is maintained after removal of the sacral and lower lumbar segments of the spinal cord even though this renders the penis and surrounding perineum anaesthetic Such cats when placed with a female in heat will develop full erection of the penis mount the female and execute copulatory movements These observations demonstrate that in rats and cats the activation of the central nervous system responsible for mating behaviour can occur in the absence of afferent impulses from the genitalia It is probable however that under normal circumstances such impulses reinforce the sex drive The studies of Beach and Holz (1946) and Beach and Levinson (1950) indicate that the effect of hormones on peripheral structures cannot be disregarded in studies of sexual behaviour Beach and Holz (1946) castrated male rats at 1 21 50 100 150 and 350 days of life After a 3 month interval all animals were tested for their response to a sexually mature female and then treatment with testosterone propionate was begun With the exception of rats castrated on the day of birth the daily injections of hormones eventually stimulated complete copulatory behaviour The males operated on the first day of life showed an increase in erotic excitability under androgen treatment but the execution of complete copulation was rare and the ejaculatory reflex occurred only once in a single individual In explanation of this fact it was found that the shaft of the penis in the day 1 group rats was shorter than that in the other groups and that normal rats in which the shaft of the penis is shortened by surgical removal of a few millimetres of the penile bone showed precisely similar behavioural patterns as this day 1 group Beach and Levinson (1950) studied the effect of testosterone on the histology of the glans penis of rats They found that normal rats and castrate animals injected with 75 μg of testosterone propionate per day possess cornified papillae in the epithelium of the glans and that adjacent to the papillae

are situated sensory corpuscles The epithelium of the glans of the castrate animal loses the papillae and becomes smooth and it is probable that such an organ is less sensitive to the stimulus of friction than the normal

It is likely that the activation of mating behaviour depends upon sensory stimuli normally derived from a multiplicity of sensory end organs Loss of one source of stimulation such as those derived from the genitalia does not necessarily impair such behaviour though it may reduce the force with which it is executed

(ii) *The effect of hormones on the integrative function of the central nervous system*

There are few descriptions in the literature of experiments in which hormones have been applied directly to the brain and found to affect the function of the nerve tissue or the behaviour of the animal Kollros (1942 1943) studied the effect of local application of thyroxine to the vicinity of the fourth ventricle in the tadpole In this animal the lid closure reflex usually appears a few days before the development of the fore limb but after the application of thyroxine in the above manner the date of appearance of the reflex was advanced Thyroxine applied to one side of the fourth ventricle stimulated the appearance of the reflex in the homolateral eye more rapidly than in the contralateral Similar experiments by Weiss and Rossetti (1951) have demonstrated an effect of locally applied thyroxine on Mauthner s cells of the brain stem of the tadpole These cells are concerned with swimming reflexes in the tadpole and disappear at the stage of metamorphosis It was found that fragments of the thyroid gland of the rat or small pieces of agar soaked in thyroxine implanted into the hindbrain of young tadpoles would cause Mauthner s cells to undergo premature atrophy though the surrounding nerve cells increased in size A similar experimental approach has rarely been used in mammalian work Kent and Liberman (1949) however studied the effect of injecting progesterone into the lateral ventricle of the brain of the hamster In their hands the ovariectomized female hamster treated with oestrogens did not develop behavioural oestrus but the intraventricular injection of small doses of progesterone in such animals resulted in mating responses The site of action of progesterone in eliciting this response is left in doubt by these experiments but from data presented below it may be tentatively suggested that the hypothalamus is the region involved Certainly progesterone injected into the lateral ventricles would be carried by the flow of cerebrospinal fluid into relationship with the hypothalamic nerve cells which lie close to the ependyma of the third ventricle

More information regarding the site of action of ovarian hormones in the central nervous system in eliciting oestrous behaviour has been acquired by studying the behavioural responses in spinal decerebrate and decorticate animals For detailed information the review by Bard (1940) should be consulted It is from this account that the following summary has been largely drawn

(*a*) *The spinal animal* Although as is well known, the autonomic aspects of copulation (erection and ejaculation) may be obtained in the spinal male animal it has not been found possible to elicit any movements (involving skeletal muscles) that resemble at all closely those normally seen in coitus

In the female mammal similar conclusions have been drawn Goltz (1874) showed that the spinal bitch may become pregnant and deliver and rear a normal litter This observation demonstrates clearly the hormonal nature of the factors controlling the reproductive organs but carries no special weight with regard to a spinal origin of mating reflexes Dempsey and Rioch (1939) and Bromiley and Bard (1940) studied chronic spinal guinea pigs and cats respectively before and after treatment with ovarian hormones Both groups of workers failed to find any suggestion of normal oestrous behaviour On the other hand Maes (1939) who studied decapitate cats concluded that some components of sexual behaviour are short arc reflexes which can be elicited independently of the higher centres The responses that Maes took as characteristic of oestrous behaviour have been criticized by Bard on the grounds that similar reactions may be obtained in anoestrous decapitate female or decapitate male cats

(*b*) *The decerebrate animal* From their studies on decerebrate guinea pigs and cats Dempsey and Rioch (1939) and Bromiley and Bard (1940) reached conclusions similar to those drawn regarding the spinal state viz that the decerebrate animal does not give any reflex response which resembles normal oestrous behaviour Maes (1939) criticized Dempsey and Rioch s failure to obtain sexual reflexes from decerebrates on the grounds that such responses were masked by the concurrent state of muscular rigidity Bromiley and Bard (1940) however found that the rigidity of their cats was not sufficient to mask alternate flexion and extension of the hind legs and alternate contraction of the

lumbar muscles of the two sides a response which was stimulated by vigorous rubbing of the vulval and perineal areas This response was not found to be related to a state of oestrus

(*c*) *The decorticate animal* Many workers have found that female mammals deprived of all neocortex will exhibit normal mating behaviour during spontaneous or induced oestrus (cats—Bard, 1934, 1936 1939 Bard and Rioch 1937 guinea pigs—Dempsey and Rioch 1939 rabbits—Brooks 1937 rats—Davis 1939) In most studies part of the rhinencephalon and corpus striatum was also removed Brooks (1937) found that female rabbits in which all neocortex and rhinencephalic cortex have been removed may mate normally but that male rabbits although mating normally after loss of the neocortex or removal of the olfactory bulbs will not mate if both these structures are removed Male rats and cats would seem to differ from male rabbits Beach (1940) found that partial loss of the neocortex in male rats reduces their ability to mate in proportion to the size (not the site) of the cortical loss and that mating activity ceases entirely if more than 60 per cent of the neocortex is removed Similar results were obtained in studies on male cats (Beach and Zitrin 1952)

From the experiments on mammals it seems clear that decorticate females may exhibit normal mating behaviour but that such behaviour is absent in the decorticate male The experiments of Brooks and Beach render it likely that even in the male there is no single structure in the cerebral cortex responsible for sexual behaviour It appears more probable that some subcortical region integrates and mediates such behaviour and that loss of the cerebral cortex prevents activation by removing the pathway of sensory stimuli Loss of the motor area of the cerebral cortex may also interfere with expression of the more active type of behaviour seen in the male

(*d*) *The hypothalamus* The experiments described above make it seem likely that some region of the brain situated above the midbrain and below the cerebral cortex is directly concerned with the integration of sexual activity What evidence is available points to the area of the upper midbrain or hypothalamus as being the region involved In acute experiments on guinea pig in induced oestrus Dempsey and Rioch (1939) found that a section which passed from the rostral end of the anterior colliculus

to a level just anterior to the mammillary bodies did not abolish oestrous responses on stimulation of the lumbar or perineal regions, but that sections made from the same point dorsally to a level just *posterior to the mammillary bodies abolished all such* responses. Magoun and Bard (1940) found that large lesions in the hypothalamus of cats rendered 'oestrus' by injections of oestradiol benzoate interfered with the oestrous behaviour of some of these animals. Since large hypothalamic lesions interfere with many general functions the effect of such lesions on sexual behaviour may not be particularly significant. Brookhart, Dey and Ranson (1940) placed smaller lesions in the hypothalamus of ovariectomized guinea pigs and found that animals with lesions near the ventral surface of the brain could not be brought into behavioural oestrus by treatment with oestrogen and progesterone. Such treatment had been found to induce full oestrus in these animals before operation. On the other hand, guinea pigs in which lesions were placed 6 mm. above the ventral surface of the brain were brought into behavioural heat by the hormonal treatment. There is some evidence also that injury to the floor of the third ventricle may result in loss of libido and potency in man (Symonds, 1943).

In an attempt to throw light on the part played by the hypothalamus in sexual behaviour, by a technique which avoided the general disturbances of hypothalamic lesions, Harris (unpublished observations) implanted minute fragments of various stilboestrol esters in different regions of the hypothalamus of ovariectomized rabbits. Some of these animals eventually showed normal mating behaviour when placed with males even though the amount of stilboestrol absorbed into the general systemic circulation was insufficient to prevent atrophy of the uterus. At the time it was thought possible that the concentration of stilboestrol in the hypothalamus had activated some neural mechanism responsible for mating behaviour, but it was found that *ovariectomized rabbits without hypothalamic stilboestrol implants* would occasionally accept the male, so that no conclusion could be drawn from these experiments. The fact that ovariectomized rabbits may accept the male has also been noted by Beach (1952*b*) and places the female rabbit in an exceptional position amongst the subprimate female mammals. Repetition of this work in some other species (such as the cat) in which the

ovariectomized female uniformly refuses the male would seem a possible avenue of investigation

The use of radioactive hormones seemed to offer another approach to the analysis of the regions of the nervous system affected by the sex hormones The following experiments (Glascock and Harris unpublished) were directed at finding whether any part of the brain accumulates oestrogen Ovariectomized rabbits that persistently refused coitus with a normal buck were injected with 25 μg labelled (tritio) hexoestrol This dose of hexoestrol contained a total of 1.7×10^{7} counts/min in the particular counting equipment used When these animals responded to the mating test they were killed and dissected Samples of muscle various regions of the brain (including the anterior and posterior region of the hypothalamus) the median eminence of the tuber cinereum anterior and posterior lobes of the pituitary mammary gland endometrium and other organs were pooled dried and assayed for radioactivity Muscle was found to assay at 6.6 counts/min /mg and no other sample except the bile was found to have an activity significantly different from this The bile contained 3 840 counts/min /mg indicating as had been expected from the results of other workers that it is one of the major routes of excretion of oestrogen The conclusion indicated by this work that oestrogens are not concentrated by regions in the hypothalamus cannot be used as evidence that they do not affect the activity of this part of the brain It seems equally possible that oestrogens may act at various sites in the brain without undergoing local concentration

The relative importance of gonadal hormones and the neocortex in activating mating behaviour

It is likely that many simple reflexes mediated by the spinal cord or brain stem are co ordinated into total patterns of mating behaviour by some upper midbrain or hypothalamic mechanism Since such behaviour is fully exhibited only during restricted periods of life the question arises by what means is the integrating region activated This is a question of some significance since the present data show that not only are different factors of importance in different species but also in the different sexes of the same species Care must then be exercised in drawing general conclusions especially in regard to human behaviour

The following table summarizes the effect of castration or removal of the neocortex on the sexual behaviour of several mammals. It is possible to say that amongst subprimate mammals female mating behaviour depends on the presence of the gonads whereas that of the female is more dependent on activation by the cerebral cortex. It is of interest that masculine behaviour on the part of female rats also seems to depend more on the presence of the neocortex (Beach, 1943), since the homosexual activity of the female rat, in contrast to the heterosexual activity of the same animal, develops before puberty, may be retained following ovariectomy but is lost after removal of the neocortex. There is little clear cut evidence regarding the activation of sexual behaviour in primates. Ford and Beach (1952) summarize the data regarding ovariectomy in primates. They state that ovariectomized adult female chimpanzees show few signs of desire for intercourse, but that ovariectomized women may show as much erotic behaviour after as before operation. These same authors state that castration of male primates often produces no diminution in the capacity to mate or (in man) to achieve sexual climax during coitus.

TABLE SUMMARISING THE EFFECT OF LOSS OF CEREBRAL CORTEX OR GONAD ON THE MATING BEHAVIOUR OF SOME MAMMALS

	Female		Male	
	Decortication	Ovariectomy	Decortication	Castration
Rat	May be unchanged (homosexual behaviour reduced)	Lost (May retain homosexual behaviour)	Lost—if more than 60 cerebral cortex removed	Lost or reduced
Cat	May be unchanged	Lost	Lost	
Dog	May be unchanged	Lost		May be unchanged
Primate	?	May be unchanged (man)	?	May be unchanged (man)

Conclusions

There is little information available as to the mechanisms by which the thyroid and adrenal cortical hormones produce changes in behaviour More work has been performed on the action of the gonadal hormones in activating mating reflexes probably because this form of activity is easily observed in common laboratory animals such as the rat and cat and because various aspects of such activity can be measured It seems likely that female mammals possess the neural basis of both homo and heterosexual behaviour which appear to depend on spinal or brain stem reflex arcs integrated into a co ordinated pattern of behaviour by some neural mechanism situated in the upper mid brain or hypothalamus If future work confirms this location it would place the region activated by gonadal hormones and responsible for mating patterns of behaviour in close conjunction with the region activated by gonadal hormones and responsible for regulating gonadotrophic secretion from the anterior pituitary gland In male carnivores and primates of both sexes the central nervous mechanism underlying mating behaviour seems to have acquired some independence from the sex hormone concentra tion in the blood stream and to be more dependent on the cere bral cortex for its activation Beach (1952*a*) has suggested that with the phylogenetic development of the cerebral cortex pat terns of sexual behaviour become more dependent on cerebral cortical activation than on chemical activation via the gonadal hormones and that this process is most highly developed in the human where there exists the most marked emancipation of erotic responsiveness from hormone control

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